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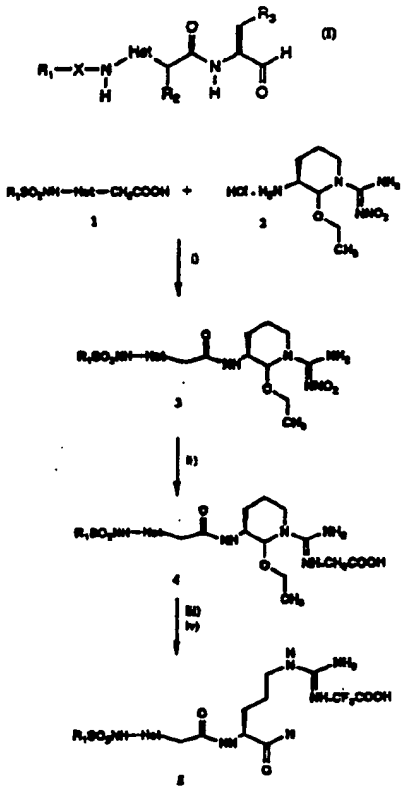
(57) Abstract

The present invention discloses peptide aldehydes of formula (I) which are potent and specific inhibitors of thrombin, their pharmaceutically acceptable salts, pharmaceutically acceptable compositions thereof, and methods of using them as therapeutic agents for disease states in mammals characterized by abnormal thrombosis.



AROMATIC HETEROCYCLIC DERIVATIVES AS ENZYME INHIBITORS

Cross Reference to Related Application

This application is a continuation-in-part of U.S.S.N. 08/481,660 and 08/484,506, both filed June 7, 1995 and both of which are continuations-in-part of U.S.S.N. 08/356,833, filed December 13, 1994; the disclosures of all these applications are incorporated herein by reference.

Technical Fields

In one aspect, the present invention relates to compounds which are potent and specific inhibitors of thrombin. In another aspect, the present invention relates to novel peptide aldehydes, their pharmaceutically acceptable salts, and pharmaceutically acceptable compositions thereof which are useful as potent and specific inhibitors of blood coagulation *in vitro* and *in vivo* in mammals. In yet another aspect, the invention relates to methods of using these inhibitors as therapeutic agents for disease states in mammals characterized by abnormal thrombosis.

Background

Normal hemostasis is the result of a complex balance between the processes of clot formation (blood coagulation) and clot dissolution (fibrinolysis). The complex interactions between blood cells, specific plasma proteins and the vascular surface, maintain the fluidity of blood unless injury and blood loss occur.

Blood coagulation is the culmination of a series of amplified reactions in which several specific zymogens of serine proteases in plasma are activated by limited

proteolysis. Nemerson, Y. and Nossel, H.L., Ann. Rev. Med., 33: 479 (1982). This series of reactions results in the formation of an insoluble fibrin matrix which is required for the stabilization of the primary hemostatic plug. The interaction and propagation of the activation reactions occurs through the extrinsic and intrinsic pathways of coagulation.

These pathways are highly inter-dependent and converge in the formation of the serine protease, Factor Xa. Factor Xa catalyzes the penultimate step in the blood coagulation cascade which is the formation of the serine protease thrombin. This step occurs following the assembly of the prothrombinase complex which is composed of factor Xa, the non-enzymatic co-factor Va and the substrate prothrombin assembled on the surface of adhered, activated platelets or systemically circulating membranous microparticles.

Proteolytic activation of zymogen factor X to its catalytically active form, factor Xa, can occur by either the intrinsic or extrinsic coagulation pathways.

The intrinsic pathway is referred to as "intrinsic" because everything needed for clotting is in the blood. Saito, H., "Normal Hemostatic Mechanisms", Disorders of Hemostasis, pp. 27-29, Grune & Stratton, Inc. (O. D. Ratnoff, M.D. and C. D. Forbes, M.D. edit. 1984). This pathway is comprised of the zymogen serine proteases, factors IX and XI, and the non-enzymatic co-factor, factor VIII. The initiation of the intrinsic pathway results in the activation of factor XI to XIa. Factor XIa catalyzes the activation of factor IX to factor IXa which in combination with the activated form of factor VIII on an appropriate phospholipid surface, results in the formation of the tenase complex. This complex also catalyzes the formation of the serine protease, factor Xa, from its zymogen, factor X which subsequently results in clot formation.

The extrinsic pathway is referred to as "extrinsic" because the tissue factor which binds to and facilitates

th activation of fact r VII comes from outside the blood. Saito, Id. The major components of this pathway are the zymogen serine protease, factor VII, and the membrane bound protein, tissue factor. The latter serves as the requisite non-enzymatic co-factor for this enzyme. The initiation of this pathway is thought to be an autocatalytic event resulting from the activation of zymogen factor VII by trace levels of activated factor VII (factor VIIa), both of which are bound to newly exposed tissue factor on membrane surfaces at sites of vascular damage. The factor VIIa/tissue factor complex directly catalyzes the formation of the serine protease, factor Xa, from its zymogen, factor X. Exposure of blood to injured tissue initiates blood clotting by the extrinsic pathway.

The formation of thrombin is catalyzed by factor Xa following the assembly of the catalytic prothrombinase complex as reviewed by Mann, K. G. et al., "Surface-Dependent Reactions of the Vitamin K-Dependent Enzyme Complexes", Blood, 76: 1-16 (1990). This complex is composed of factor Xa, the non-enzymatic co-factor Va and the substrate prothrombin all assembled on an appropriate phospholipid surface. The requirement of a macromolecular complex for efficient catalysis results in the protection of factor Xa from natural anticoagulant mechanisms such as heparin-antithrombin III mediated inhibition. Teite, J. M. and Rosenberg, R. D., "Protection of Factor Xa from neutralization by the heparin-antithrombin complex", J. Clin. Invest., 71: 1383-1391(1983). In addition, sequestration of factor Xa in the prothrombinase complex also renders it resistant to inhibition by exogenous heparin therapy which also requires antithrombin III to elicit its anticoagulant effect.

Thrombin is the primary mediator of thrombus formation. Thrombin acts directly to cause formation of insoluble fibrin from circulating fibrinogen. In addition, thrombin activates the zymogen factor XIII to the active transglutaminase factor XIIIa which acts to covalently stabilize the growing thrombus by crosslinking

th fibrin strands. Lorand, L. and Konishi, K., Arch. Biochem. Biophys., 105: 58 (1964). Beyond its direct role in the formation and stabilization of fibrin rich clots, the enzyme has been reported to have profound bioregulatory effects on a number of cellular components within the vasculature and blood. Shuman, M.A., Ann. NY Acad. Sci., 405: 349 (1986).

It is believed that thrombin is the most potent agonist of platelet activation, and it has been demonstrated to be the primary pathophysiologic-mediator of platelet-dependent arterial thrombus formation. Edit, J.F. et al., J. Clin. Invest., 84: 18 (1989). Thrombin-mediated platelet activation leads to ligand-induced inter-platelet aggregation principally due to the bivalent interactions between adhesive ligands such as fibrinogen and fibronectin with platelet integrin receptors such as glycoprotein IIb/IIIa which assume their active conformation following thrombin activation. Berndt, M.C. and Phillips, D.R., Platelets in Biology and Pathology, pp 43-74, Elsevier/North Holland Biomedical Press (Gordon, J.L. edit. 1981). Thrombin-activated platelets can also support further thrombin production through the assembly of new prothrombinase and tenase (factor IXa, factor VIIIa and factor X) catalytic complexes on the membrane surface of intact activated platelets and platelet-derived microparticles, following thrombin-mediated activation of the non-enzymatic cofactors V and VIII, respectively. Tans, G. et al., Blood, 77: 2641 (1991). This positive feedback process results in the local generation of large concentrations of thrombin within the vicinity of the thrombus which supports further thrombus growth and extension. Mann, K.G. et al., Blood, 76: 1 (1990).

In contrast to its prothrombotic effects, thrombin has been shown to influence other aspects of hemostasis. These include its effect as an important physiological anticoagulant. The anticoagulant effect of thrombin is expressed following binding of thrombin to the endothelial cell membrane glycoprotein, thrombomodulin. This is

thought to result in an alteration of the substrate specificity of thrombin thereby allowing it to recognize and proteolytically activate circulating protein C to give activated protein C (aPC). Musci, G. et al., Biochemistry, 27: 769 (1988). aPC is a serine protease which selectively inactivates the non-enzymatic co-factors Va and VIIIa resulting in a down-regulation of thrombin formation by the prothrombinase and tenase catalytic complexes, respectively. Esmon, C.T., Science, 235: 1348 (1987). The activation of protein C by thrombin in the absence of thrombomodulin is poor.

Thrombin has also been shown to be a potent direct mitogen for a number of cell types, including cells of mesenchymal origin such as vascular smooth muscle cells. Chen, L.B. and Buchanan, J.M., Proc. Natl. Acad. Sci. USA, 72: 131 (1975). The direct interaction of thrombin with vascular smooth muscle also results in vasoconstriction. Walz, D.A. et al., Proc. Soc. Expl. Biol. Med., 180: 518 (1985). Thrombin acts as a direct secretagogue inducing the release of a number of bioactive substances from vascular endothelial cells including tissue plasminogen activator. Levin, E.G. et al., Thromb. Haemost., 56: 115 (1986). In addition to these direct effects on vascular cells, the enzyme can indirectly elaborate potent mitogenic activity on vascular smooth muscle cells by the release of several potent growth factors (*e.g.*, platelet-derived growth factor and epidermal growth factor) from platelet a-granules following thrombin-induced activation. Ross, R., N. Engl. J. Med., 314: 408 (1986).

Many significant disease states are related to abnormal hemostasis. With respect to the coronary arterial vasculature, abnormal thrombus formation due to the rupture of an established atherosclerotic plaque is the major cause of acute myocardial infarction and unstable angina. Moreover, treatment of an occlusive coronary thrombus by either thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA) is often accompanied by an acute thrombotic reclosure of the

affect d vessel which requires immediate resolution. With respect to the venous vasculature, a high percentage of patients undergoing major surgery in the lower extremities or the abdominal area suffer from thrombus formation in the venous vasculature which can result in reduced blood flow to the affected extremity and a predisposition to pulmonary embolism. Disseminated intravascular coagulopathy commonly occurs within both vascular systems during septic shock, certain viral infections and cancer and is characterized by the rapid consumption of coagulation factors and systemic coagulation which results in the formation of life-threatening thrombi occurring throughout the vasculature leading to widespread organ failure.

Pathogenic thrombosis in the arterial vasculature is a major clinical concern in today's medicine. It is the leading cause of acute myocardial infarction which is one of the leading causes of death in the western world. Recurrent arterial thrombosis also remains one of the leading causes of failure following enzymatic or mechanical recanalization of occluded coronary vessels using thrombolytic agents or percutaneous transluminal coronary angioplasty (PTCA), respectively. Ross, A.M., Thrombosis in Cardiovascular Disorder, p. 327, W.B. Saunders Co. (Fuster, V. and Verstraete, M. edit. 1991); Califf, R.M. and Willerson, J.T., Id. at p 389. In contrast to thrombotic events in the venous vasculature, arterial thrombosis is the result of a complex interaction between fibrin formation resulting from the blood coagulation cascade and cellular components, particularly platelets, which make up a large percentage of arterial thrombi. Heparin, the most widely used clinical anticoagulant administered i.v., has not been shown to be universally effective in the treatment or prevention of acute arterial thrombosis or rethrombosis. Prins, M.H. and Hirsh, J., J. Am. Coll. Cardiol., 67: 3A (1991).

Besides the unpredictable, recurrent thrombotic reocclusion which commonly occurs following PTCA, a

profound restenosis of the recanalized vessel occurs in 30 to 40% of patients 1 to 6 months following this procedure. Califf, R.M. et al., J. Am. Coll. Cardiol., 17: 2B (1991). These patients require further treatment with either a repeat PTCA or coronary artery bypass surgery to relieve the newly formed stenosis. Restenosis of a mechanically damaged vessel is not a thrombotic process but instead is the result of a hyperproliferative response in the surrounding smooth muscle cells which over time results in a decreased luminal diameter of the affected vessel due to increased muscle mass. Id. As for arterial thrombosis, there is currently no effective pharmacologic treatment for the prevention of vascular restenosis following mechanical recanalization.

The need for safe and effective therapeutic anticoagulants has in one aspect focused on the role of the serine protease thrombin in blood coagulation.

Most preferred natural substrates for thrombin are reported to contain an uncharged amino acid in the P3 recognition subsite. For example, the thrombin cleavage site on the Aα chain of fibrinogen, which is the primary physiological substrate for thrombin, is reported to contain a glycine residue in this position while the cleavage site on the Bβ chain contains a serine, as shown below:

P4	P3	P2	P1	P1'	
Gly	Gly	Val	Arg	Gly	Fibrinogen Aα Chain
Phe	Ser	Ala	Arg	Gly	Fibrinogen Bβ Chain

Peptidyl derivatives having an uncharged residue in the P3 position are said to bind to the active site of thrombin and thereby inhibit the conversion of fibrinogen to fibrin and inhibit cellular activation. These derivatives have either an aldehyde, chloromethyl ketone or boronic acid functionality associated with the P1 amino acid. For example, substrate-like peptidyl derivatives such as D-phenylalanyl-prolyl-argininal (D-Phe-Pro-Arg-al), D-phenylalanyl-prolyl-arginine-chloromethyl ketone

(P-PACK) and acetyl-D-phenylalanyl-prolyl-boroarginine (Ac-(D-Phe)-Pro-boroArg) have been reported to inhibit thrombin by directly binding to the active site of the enzyme. Bajusz, S., Symposia Biologica Hungarica, 25: 277 (1984), Bajusz, S. et al, J. Med. Chem., 33: 1729 (1990) and Bajusz, S. et al., Int. J. Peptide Protein Res. 12: 217 (1970); Kettner, C. and Shaw, E., Methods Enzymol., 80: 826 (1987), Kettner, C. et al., EP 293,881 (published December 7, 1988), Kettner, C., et al., J. Biol. Chem., 265: 18209 (1990). These molecules have been reported to be potent anticoagulants in the prevention of platelet-rich arterial thrombosis. Kelly, A.B. et al., Thromb. Haemostas., 65: 736 at abstract 257 (1991). Other peptidyl aldehydes have been proposed or reported as inhibitors of thrombin. Bey, P. et al., EP 363,284 (published April 11, 1990) and Balasubramanian, N. et al., EP 526,877 (published February 10, 1993).

Peptidyl compounds which are said to be active site inhibitors of thrombin but which differ in structure from those containing a uncharged amino acid in the P3 recognition subsite have been reported.

The compound, Argatroban (also called 2R,4R-4-methyl-1-[N-2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-argininal]-2-piperdinecarboxylic acid), is also reported to bind directly to the active site of thrombin and has been thought to be the most potent and selective compound in the class of non-peptidyl inhibitors of this enzyme. Okamoto, S. et al., Biochem. Biophys. Res. Commun., 101: 440 (1981). Argatroban has been reported to be a potent antithrombotic agent in several experimental models of acute arterial thrombosis. Jang, I.K. et al., in both Circulation, 81: 219 (1990) and Circ. Res., 67: 1552 (1990).

Peptidyl compounds which are said to be inhibitors of thrombin and whose mode of action is thought to be by binding to both the active site and another site on the enzyme have been reported. Hirudin and certain peptidyl derivatives of hirudin have been r ported to inhibit both

conversion of fibrinogen to fibrin and platelet activati n by binding to either both the active site and exo site, or the exo site only, of thrombin. Markwardt, F., Thromb. Haemostas., 66: 141 (1991). Hirudin is reported to be a 65 amino acid polypeptide originally isolated from leech salivary gland extracts. It is said to be one of the most potent inhibitors of thrombin known. Marki, W.E. and Wallis, R.B., Thromb. Haemostas., 64: 344 (1990). It has been reported to inhibit thrombin by binding to both its anion-binding exo-site and to its catalytic active site which are distinct and physically distant from each other. Rydel, T.J. et al., Science, 249:277 (1990). Hirudin has been reported to be a potent antithrombotic agent *in vivo*. Markwardt, F. et al., Pharmazie, 43: 202 (1988); Kelly, A.B. et al., Blood, 77: 1 (1991). In addition to its antithrombotic effects, hirudin has been reported to also effectively inhibit smooth muscle proliferation and the associated restenosis following mechanical damage to a atherosclerotic rabbit femoral artery. Sarembock, I.J. et al., Circulation, 84: 232 (1991).

Hirugen has been reported to be a peptide derived from the anionic carboxy-terminus of hirudin. It is reported to bind only to the anion binding exo-site of thrombin and thereby inhibit the formation of fibrin but not the catalytic turnover of small synthetic substrates which have access to the unblocked active site of the enzyme. Maraganore, J.M. et al., J. Biol. Chem., 264: 8692 (1989); Naski, M.C. et al., J. Biol. Chem., 265: 13484 (1990). Based on x-ray crystallographic analysis, it has been reported that the region of hirudin represented by hirugen binds directly to the exo site of thrombin. Skrzypczak-Jankun, E. et al., Thromb. Haemostas., 65: 830 at abstract 507 (1991). Moreover, the binding of hirugen has also been reported to enhance the catalytic turnover of certain small synthetic substrates by thrombin, indicating that a conformational change in the enzyme active sit may accompany occupancy of the exo-site. Liu, L.W. et al., J. Biol. Chem, 266:16977 (1991).

Hirugen also is reported to block thrombin-mediated platelet aggregation. Jakubowski, J.A. and Maraganore, J.M., Blood, 75: 399 (1990).

A group of synthetic chimeric molecules comprised of a hirugen-like sequence linked by a glycine-spacer region to the peptide, D-phenylalanyl-prolyl-arginine, which is based on a preferred substrate recognition site for thrombin, has been termed to be hirulog. Maraganore et al., U.S. Patent No. 5,196,404 (March 23, 1993). The hirugen-like sequence is said to be linked to this peptide through the C-terminal end of the peptide. Maraganone, J.M. et al., Biochemistry, 29: 7095 (1990). The hirulogs have been reported to be an effective antithrombotic agents in preventing both fibrin-rich and platelet-rich thrombosis. Maraganone, J.M. et al., Thromb. Haemostas., 65: 651 at abstract 17 (1991).

Certain benzamidines have been reported to inhibit thrombin though non-selectively. 4-amidinophenylpyruvic acid (APPA) has been reported to be a thrombin inhibitor with low toxicity and favorable pharmacokinetics. However, this compound was reported to be non-selective, inhibiting trypsin, plasmin and kallikrein. Markwardt et al., Thromb. Res., 1:243-52 (1972). Other benzamidine-derived structures which have been reported to inhibit thrombin include the cylic amides of N^a-substituted 4-amidinophenylalanine and 2-amino-5-(4-amidinophenyl)-1-valeric acid. The inhibitory constant displayed by these compounds was reported to be in the micromolar range. Markwardt et al., Thromb. Res., 17:425-31 (1980). Moreover, derivatives of 4-amidinophenylalanine whose a-amino group is linked to the arylsulfonyl residue via an w-aminoalkylcarboxylic acid as spacer have also been assessed for their inhibitory effect. Among these N^a-(2-naphthylsulphonylglycyl)-4-amidino-phenylalanine piperidide (a-NAPAP) has been reported to possess an affinity for thrombin (K_i=6 x 10^{-9} M). Banner et al., J. Biol. Chem., 266:20085 (1991) and Sturzebecher et al., Thromb. Res., 29:635-42 (1983).

Certain bis-benzamidines have been reported to inhibit thrombin. The antithrombin activity of bis-benzamidines was reported to increase with the length and bulkiness of the central chain. However, these compounds were reported to be generally toxic in the micromolar range where they are also inhibitory. Geratz et al., Thromb. Diath. Haemorrh., 29:154-67 (1973); Geratz et al., J. Med. Chem., 16:970-5 (1973); Geratz et al., J. Med. Chem., 19:634-9 (1976); Walsmann et al., Acta Biol. Med. Germ., 35:K1-8 (1976); and Hauptmann et al., Acta Biol. Med. Germ., 35:635-44 (1976).

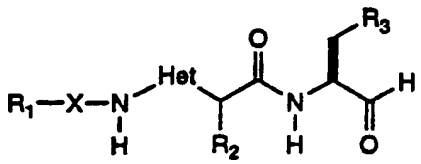
Certain amidino-bearing aromatic ring structures such as beta-naphthamidines have been reported to possess modest antithrombin and anticoagulant activity. This class of compounds include the non-selective 6-amidino-2-naphthyl-4-guanidinobenzoate dimethanesulfonate (FUT 175). Fuji et al., Biochim. Biophys. Acta, 661:342-5 (1981); and Hitomi et al., Haemostasis, 15:164-8 (1985).

Certain phenylguanidines have been reported to inhibit thrombin. Derivatives of 4-guanidinophenylalanine with inhibitory constants in the micromolar range have been reported to inhibit thrombin. This class includes the N^a-tosylated and dansylated 4-guanidino phenylalanine piperidides. Claeson et al., Thromb. Haemostas., 50:53 (1983). Another compound, [ethyl p-(6-guanidinohexanoyloxy) benzoate] methane sulfonate (FOY) was reported to be a non-selective competitive inhibitor of thrombin. Ohno et al., Thromb. Res., 19:579-588 (1980).

Summary of the Invention

The present invention is directed to novel peptide aldehyde compounds having arginine or arginine mimics at P_1 and pyridone, pyrimidone, or uracil groups as part of the peptide backbone. These compounds are potent inhibit rs of thrombin *in vivo* and *in vitro*.

Thus, in one aspect, the present invention is directed to compounds of the formula:



(I)

wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R")-$ and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and;

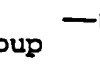
(b) R_1 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the ring carbons with hydroxyl, alkoxyl or alkyl each of 1 to about 3 carbons, amino, guanidino, or amidino,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, including the group  , wherein  is a 5 to 7 member heterocycl of 3 to 6 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, and which is

optionally substituted on the ring carbons with hydroxyl, alkoxyl or alkyl each of 1 to about 3 carbons, amino, guanidino, or amidino,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl of 1 to about 3 carbons,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

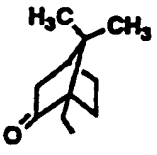
(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

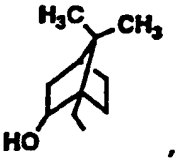
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,

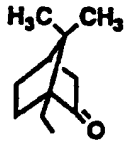
(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

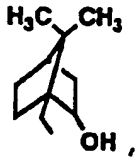
(11) aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

(17) difluoromethyl and perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(20) hydrogen,

wherein Y_1, Y_2, and Y_3 are

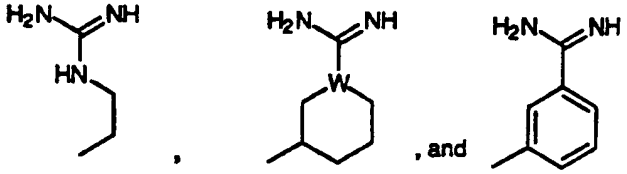
(i) independently selected from the group consisting of hydrogen, halogen, cyano, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2H$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)OZ_1$, $-P(O)_3H$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, and $-NZ_1Z_2$, wherein m is 0, 1 or 2, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14

carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen,

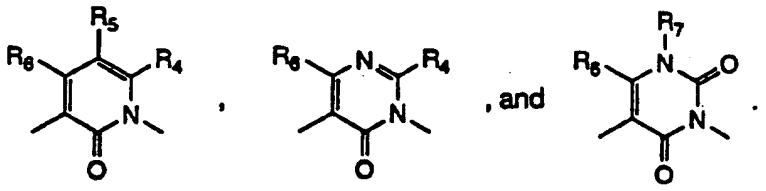
(c) R_2 is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms, and alkenyl of about 2 to about 4 carbon atoms,

(d) R_3 is selected from the group consisting of



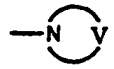
where W is nitrogen or carbon;

(e) Het is selected from the group consisting of



wherein

(1) R_4 is selected from the group consisting of

(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, and halogen, wherein n is 0, 1 or 2, and R_1 is independently selected and as defined above, with the proviso that R_4 is not a camphor derivative or  heterocyclo group,

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_5 wherein Z_5 is selected from the group

consisting of hydroxy, halogen, -C(O)OH, -C(O)OR_8, -S(O)$_3$OH, and -S(O)$_p R_8$ wherein R_8 is alkyl of 1 to about 6 carbon atoms and p is 0, 1 or 2, and

(c) alkenyl of about 3 to about 6 carbon atoms;

(2) R_5 is selected from the group consisting of

(a) hydrogen,

(b) alkyl of 1 to about 10 carbon atoms,

(c) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms,

(d) cyclic alkyl of 3 to about 6 carbon atoms,

(e) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and -S(O)$_i$- wherein i is independently 0, 1 or 2,

(f) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and -S(O)$_i$- wherein i is independently 0, 1 or 2 and which is attached to Het by a ring carbon atom,

(g) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of 3 to about 5 carbon atoms,

(h) aryl which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3 respectively,

(i) heteroaryl of 5 to 6 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and -S(O)$_i$- wherein i is independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3,

(j) aralkyl of about 7 to about 10 carbon atoms which is optionally mono-, di- or tri-substituted on the aryl ring with Y_1, Y_2 and/or Y_3;

(k) heteroaralkyl of 6 to 9 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted on the ring with Y_1, Y_2 and/or Y_3,

(l) aralkenyl of 8 carbon atoms which is optionally mono-, di- or tri- substituted on the aryl ring with Y_1, Y_2 and/or Y_3,

(m) heteroaralkenyl of 7 to 8 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $-S(O)_i-$ wherein i is independently 0, 1 or 2, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3,

(n) halogen,

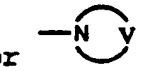
(o) difluoromethyl or perfluoroalkyl of 1 to 3 carbon atoms,

(p) perfluorophenyl,

(q) perfluoroaralkyl of 7 to about 9 carbon atoms, and

(r) alkoxy of 1 to about 10 carbon atoms;

(3) R_6 is selected from the group consisting of

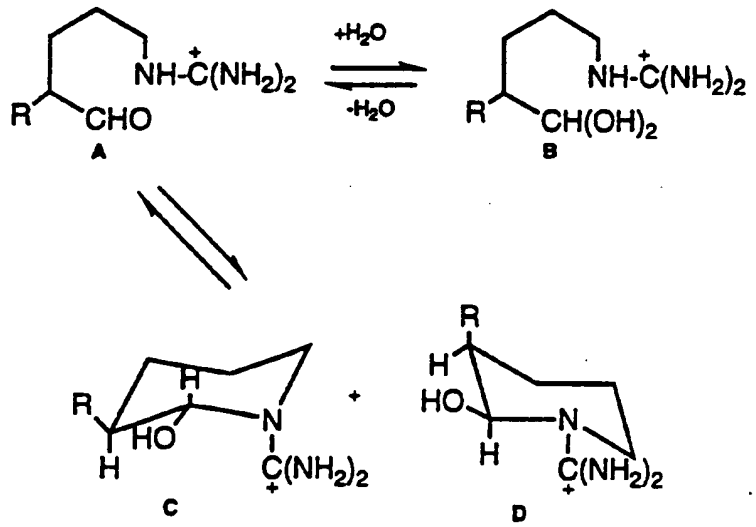
(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, and halogen, wherein n is 0, 1 or 2, and R_1 is independently selected and as defined above, with the proviso that R_6 is not a camphor derivative or  heterocyclo group, and

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, $-OR_9$, $-NHR_9$, $-C(O)OH$, $-C(O)OR_9$, $-S(O)_2OH$ and $-S(O)_pR_9$ wherein R_9 is selected from alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 10 carbon atoms optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 12 carbon atoms optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3,

heteroaryl of 1 to about 9 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and heteroaralkyl of about 2 to about 10 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and

(4) R_7 is independently selected from the R_5 group of substituents, provided that R_7 is not halogen; and pharmaceutically acceptable salts thereof.

Peptidyl arginine aldehydes have been reported to exist in equilibrium structures in aqueous solutions. Bajusz, S., et al., J. Med. Chem., 33: 1729 (1990). These structures, as shown below, include the arginine aldehyde, A, aldehyde hydrate, B, and two amino cyclol forms, C and D. The R group would represent the remainder of a given compound embodied in the present invention. The peptide aldehydes of the present invention include within their definition all the equilibrium forms.



Among other factors, the present invention is based on ur finding that the novel compounds of our invention are active as selective inhibitors of thrombin. In particular, we have found that certain of the preferred

compounds of the present invention exhibit advantag us selectivity in that they are very potent inhibitors of thrombin but are inactive or significantly less active, (several orders of magnitude less) in inhibiting plasmin and are significantly less active in inhibiting trypsin. This selectivity for inhibition of thrombin gives these compounds a therapeutic advantage in treating or preventing thrombosis in a mammal suspected of having a condition characterized by abnormal thrombosis.

In another aspect, the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a compound of the present invention and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention is directed to methods of using the compounds and pharmaceutical compositions of the present invention for the prevention of thrombosis in a mammal suspected of having a condition characterized by abnormal thrombosis, comprising administering to said mammal a therapeutically effective amount of a compound of the present invention or pharmaceutical composition comprising such a compound.

Definitions

In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic groups.

The terms "alkoxy" and "alkoxyl" refer to a group having the formula, R-O-, wherein R is an alkyl group.

The term "alkoxycarbonyl" refers to -C(O)OR wherein R is alkyl.

The term "aralkenyl" refers to an alkenyl group substituted with an aryl group.

The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups

include benzyl, picolyl, and the like, all of which may be optionally substituted.

The term "aryl" refers to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

The term "aryloxy" refers to a group having the formula, R-O-, wherein R is an aryl group.

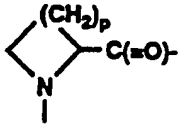
The term "aralkoxy" refers to a group having the formula, R-O-, wherein R is an aralkyl group.

The term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4 diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone,

S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) -C(O)-R-NH-, wherein R typically is -CH(R')-, wherein R' is H or a carbon containing

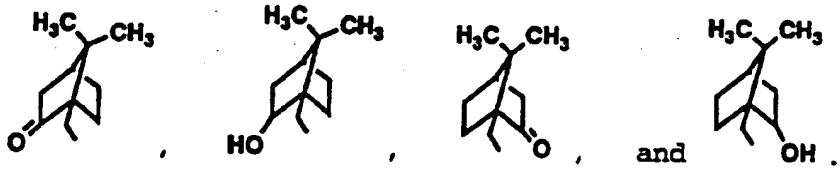


substituent; or (2) , wherein p is 1, 2 or 3 representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

"Biaryl" refers to phenyl substituted by carbocyclic or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring.

"Brine" refers to an aqueous saturated solution of sodium chloride.

"Camphor derivative" refers to the groups:



"Carbocyclic aryl" refers to aromatic groups wherein the ring atoms of the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups, such as phenyl; naphthyl and other polycyclic groups, all of which may be optionally substituted. Suitable carbocyclic aryl groups include phenyl and naphthyl. Suitable substituted carbocyclic aryl groups include indene and phenyl substituted by one to two

substituents such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkoxycarbonyl, halogen, trifluoromethyl, nitro, and cyano. Substituted naphthyl refers to 1- or 2-naphthyl substituted by lower alkyl, lower alkoxy, or halogen.

"Cycloalkenyl" refers to a cyclic alkenyl group. Suitable cycloalkenyl groups include, for example, cyclopentenyl and cyclohexenyl.

"Cycloalkyl" refers to a cyclic alkyl group. Suitable cycloalkyl groups include, for example, cyclohexyl, cyclopropyl, cyclopentyl, and cycloheptyl.

"Cyclohexylmethyl" refers to a cyclohexyl group attached to CH_2.

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

"Heteroaralkenyl" refers to an alkenyl group substitued with a heteroaryl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

"Heteroaralkyl" refers to an alkyl group substituted with a heteroaryl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

"Heteroaryl" refers to aryl groups having from 1 to 9 carbon atoms and the remainder of the atoms are heteroatoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Suitable heteroatoms include oxygen, nitrogen, $S(O)_i$, wherein i is 0, 1 or 2, and suitable heterocyclic

aryls include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

"Heterocyclo" refers to a reduced heterocyclic ring system comprised of carbon, nitrogen, oxygen and/or sulfur atoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

"Heterocycloalkyl" refers to an alkyl group substituted with a heterocyclo group, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

The term "lower" referred to herein in connection with organic radicals or compounds defines such with up to and including 5, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched chain.

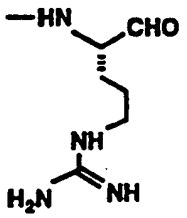
"Perfluoroalkyl" refers to an alkyl group which has every hydrogen replaced with fluorine.

"Perfluoroaryl" refers to an aryl group which has every hydrogen replaced with fluorine.

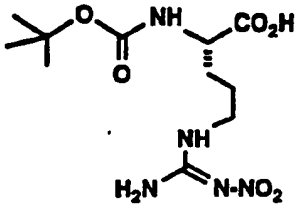
"Perfluoroaryl alkyl" refers an aralkyl group in which every hydrogen on the aryl moiety is replaced with fluorine.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

The term "Arg-al" refers to the residue of L-argininal which has the formula:



"N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine" refers to the compound which has the formula:



In addition, the following abbreviations stand for the following:

"Boc" or "BOC" refers to t-butoxycarbonyl.

"BOP" refers to benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate.

"$BzlSO_2$" refers to benzylsulfonyl.

"DCC" refers to N,N'-dicyclohexylcarbodiimide.

"EDC" refers to 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt.

"HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

"HCl" refers to hydrochloric acid.

"HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"HPLC" refers to high pressure liquid chromatography.

"2-PrPen" refers to 2-propylpentanoyl.

"$LiAlH_4$" refers to lithium aluminum hydride.

"$LiAlH_2(OEt)_2$ refers to lithium aluminum dihydride diethoxide.

"NaOH" refers to sodium hydroxide.

"NMM" refers to N-methylmorpholine.

"TBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

"THF" refers to tetrahydrofuran.

"TLC" refers to thin layer chromatography.

Brief Description of the Drawings

Figure 1 depicts a general reaction scheme for preparation of certain compounds of the present invention. In this figure, i) through iv) are defined as: i) N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt and N-methylmorpholine; ii) hydrogen gas, palladium on carbon, ethanol, acetic acid and water; iii) 3 N HCl; and iv) sodium acetate, followed by HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 2 depicts a general reaction scheme for preparation of certain compounds of the present invention. In this figure, I) through viii) are defined as: i) sodium hydride and ethyl bromoacetate; ii) hydrogen gas and palladium on carbon; iii) collidine and R_1-SO_2-Cl, where R_1 is as defined herein; iv) aqueous sodium hydroxide and methanol; v) N^g-nitro-L-argininal ethyl cyclol, hydrochloride salt, N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt and N-methylmorpholine; vi) hydrogen gas, palladium on carbon, ethanol, acetic acid and water; vii) 3N HCl; and viii) sodium acetate, and then HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 3 depicts a general reaction scheme for preparation of certain compounds of the present invention. In this figure, I) through xiv) are defined as: i) R_4-C(=NH)-NH_2, where R_4 is as defined herein; ii) sodium hydride, allyl bromide; iii) sodium hydroxide; iv) triethylamine, diphenylphosphoryl azide and heat; v) t-butyl alcohol and heat; vi) trifluoroacetic acid; vii) collidine and R_1-SO_2-Cl, where R_1 is as defined herein; viii) N-methylmorpholine-N-oxide and osmium tetroxide; ix) sodium periodate; x) sodium chlorite; xi) N^g-nitro-L-

argininal ethyl cycl 1, hydrochloride salt, N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt and N-methylmorpholine; xii) palladium on carbon, ethanol, acetic acid and water; xiii) 3N HCl; and xiv) sodium acetate, and then HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 4 depicts a general reaction scheme for preparation of certain compounds of the present invention. In this figure, i) though x) are defined as: i) 1,1,1-3,3,3-hexamethyldisilazane and chlorotrimethylsilane; ii) R_7X heated in dimethylformamide, wherein R_7 is as defined herein and X is a halogen; iii) tetrabutylammonium fluoride and ethyl bromoacetate; iv) hydrogen gas and palladium on carbon; v)collidine and R_1-SO_2-Cl, where R_1 is as defined herein; vi) sodium hydroxide; vii) N^g-nitro-L-argininal ethyl cyclol, hydrochloride salt, N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt and N-methylmorpholine; viii) palladium on carbon, ethanol, acetic acid and water; ix) 3N HCl; and x) sodium acetate, and then HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 5 depicts the reaction scheme for preparation of a compound of the present invention. In this figure, "Boc" refers to the protecting group, t-butoxycarbonyl; "Cbz" refers to the protecting group, benzyloxycarbonyl; and "t-Bu" refers to the protecting group, t-butyl. Also, in this figure, i) through x) are defined as: i) lithium aluminum hydride; ii) ethanol and HCl; iii) hydrogen gas and palladium on carbon, 1.0N HCl; iv) sodium hydride and t-butyl bromoacetate; v) hydrogen gas and palladium on carbon; vi) sodium bicarbonate and allyl chloroformate; vii) trifluoroacetic acid; viii) N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt and N-methylmorpholine; ix) hexafluorophosphoric acid; and x) sodium acetate and then

HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 6 depicts the anticoagulant effect of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl] acetyl-L-arginal measured in citrated human plasma, closed circles (l), using the activated partial thromboplastin time (APTT) assay. The control clotting times (0 inhibitor) for human plasma was 29 seconds. The concentration of this compound of the present invention which caused a doubling of the control clotting time in human plasma was 7.7 micromolar. The data is the mean of two independent determinations.

Figure 7 depicts a reaction scheme for the preparation of compounds wherein X is a direct link. In this figure, i) through ix) are defined as i) hydrogen gas and palladium on carbon; ii) di-t-butyldicarbonate and sodium bicarbonate; iii) sodium hydride and R_1 iodide; iv) sodium hydroxide; v) EDC, HOBt, and N-methylmorpholine; vi) hydrogen gas and palladium on carbon, ethanol, acetic acid and water; vii) 3N hydrochloric acid; and viii) sodium acetate, and then HPLC purification using 0.1% trifluoroacetic acid in acetonitrile and water.

Figure 8 depicts a reaction scheme for the preparation of certain compounds of the present invention. In this Figure, i) through vi) are: i) potassium carbonate, dimethylformamide and t-butyl bromoacetate; ii) lithium hydroxide and tetrahydrofuran; iii) triethylamine, diphenylphosphoryl azide, dioxane and heat (about __°C); benzyl alcohol and Δ; iv) hydrogen gas and palladium on carbon; v) R_1SO_2Cl and collidine; and vi) trifluoroacetic acid. See also Examples 91-96. R_1 is as defined in connnection with formula (I) herein.

Figure 9 depicts a reaction scheme for the preparation of certain compounds of the present invention, including compound **18** in Figure 3. In this Figure, i) through vi) are: i) tetra-n-butyl ammonium fluoride, dimethoxyethane, and t-butyl bromoacetate; ii) lithium hydroxide and tetrahydrofuran; iii) triethylamine,

diphenylphosphorylazide, dioxane and Δ; benzyl alcohol and Δ; iv) hydrogen gas and palladium on carbon; v) R_1SO_2Cl and 4-methylmorpholine and vi) trifluoroacetic acid. See also Examples 102-107. R_1 and R_4 are as defined in connection with formula (I) herein.

Figure 10 depicts a reaction scheme for the preparation of certain compounds of the present invention, including compound **26** in Figure 4. In this Figure, i) through v) are: i) potassium carbonate, R_7X and dimethylsulfoxide; ii) sodium hydride and t-butylbromoacetate; iii) hydrogen gas and 10% palladium on carbon; iv) R_1SO_2Cl and 4-methylmorpholine; and v) trifluoroacetic acid. See also Examples 108-111. R_1 and R_7 are as defined in connection with formula (I) herein.

Figure 11 depicts a reaction scheme for the preparation of certain compounds of the present invention. In this Figure, i) through vii) are: i) 2 equivalents, lithium diisoprophylamide, R_xX, 50% sulfuric acid; iii) triethylamine, diphenylphosphonyl azide, dioxane and Δ; benzyl alcohol and Δ; iv) sodium hydride, dimethylformamide and t-butyl bromoacetate; v) hydrogen gas and palladium on carbon; vi) R_1SO_2Cl and collidine; and vii) trifluoroacetic acid. See also Examples 97-101. R_1 is as defined in connection with formula (I) herein. R_x is any R_4 substituent minus one carbon, such as methyl if R_1 was ethyl and X is halogen.

Figure 12 depicts a reaction scheme for the preparation of certain compounds of the present invention. In this Figure, i) through iv) are: i) lithium hexamethyldisilazide, chlorotrimethylsilane, lithium hexamethyldisilazide and benzaldehyde; ii) lithium hexamethyldisilazide and ethyl bromoacetate; iii) acetic anhydride, 10% palladium on carbon, hydrogen gas; and iv) lithium hydroxide. See also Examples 114-116.

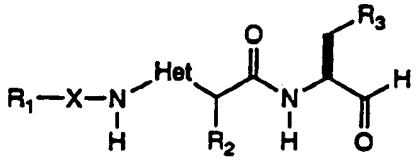
Figure 13 depicts a reaction scheme for the preparation of certain compounds of the present invention. In this Figure, i) through ix) are: i) thiourea and methanol to give a 97% yield of **72** (compound of Example

135); ii) chlorine gas, water to give 90% yield of 73 (compound of Example 136); iii) lithium bis(trimethylsilyl)amide, tetrahydrofuran and t-butyl bromoacetate to give 75% yield of 75 (compound of Example 137); iv) hydrogen gas and palladium on carbon to give 98.5% yield of 76 (compound of Example 138); v) 4-methylmorpholine, and acetonitrile to give 65% yield of 78 (compound of Example 139); vi) trifluoroacetic acid and methylene chloride to give a quantitative yield of 79 (compound of Example 140). vii) N-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt, 80 (N^{g}-nitro-L-argininal ethyl cyclol), 4-methylmorpholine at room temperature to give 55% yield of 81 (compound of Example 141). viii) hydrogen gas (1 atm), palladium on carbon, ethanol, acetic acid to give quantitative yield of 82 (compound of Example 142); and ix) 6N HCl, room termperature, about one hour to give 74% yield of 83 (compound of Example 143). See also Examples 135 to 143.

Detailed Description of the Invention

1. Preferred Compounds

Compounds of the present invention have the formula:



wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R")-$ and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and;

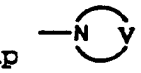
(b) R_1 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the ring carbons with hydroxyl, alkoxyl or alkyl each of 1 to about 3 carbons, amino, guanidino, or amidino,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, including the group  , wherein  is a 5 to 7 member heterocycle of 3 to 6 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, and which is optionally substituted on the ring carbons with hydroxyl, alkoxyl, or alkyl each of 1 to about 3 carbons, amino, guanidino, or amidino,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$,

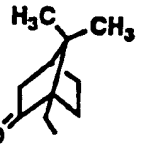
wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,

(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,

(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

(13) 

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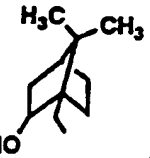
(14) 

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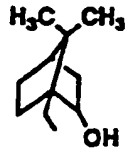
(16) 

,

(17) difluoromethyl and perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(20) hydrogen,

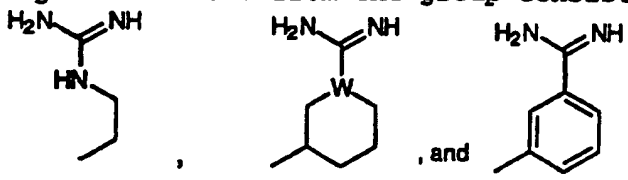
wherein Y_1, Y_2, and Y_3 are

(i) independently selected from the group consisting of hydrogen, halogen, cyano, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2H$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)OZ_1$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, and $-NZ_1Z_2$, wherein m is 0, 1 or 2, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen;

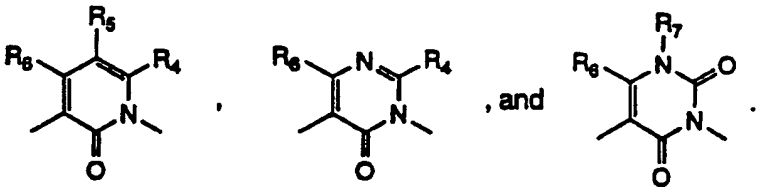
(c) R_2 is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms, and alkenyl of 2 to about 4 carbon atoms,

(d) R_3 is selected from the group consisting of



where W is nitrogen or carbon;

(e) Het is selected from the group consisting of



wherein

(1) R_4 is selected from the group consisting of

(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, and halogen, wherein n is 0, 1 or 2, and R_1 is independently selected and as defined above, with the proviso that R_4 is not a camphor derivative or  heterocyclo group,

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_5 wherein Z_5 is selected from the group consisting of hydroxy, halogen, $-C(O)OH$, $-C(O)OR_8$, $-S(O)_3OH$, and $-S(O)_pR_8$ wherein R_8 is alkyl of 1 to about 6 carbon atoms and p is 0, 1 or 2, and

(c) alkenyl of about 3 to about 6 carbon atoms;

(2) R_5 is selected from the group consisting of

(a) hydrogen,

(b) alkyl of 1 to about 10 carbon atoms,

(c) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms,

(d) cyclic alkyl of 3 to about 6 carbon atoms,

(e) heterocycloalkyl of 4 to about 6 ring

atoms with th ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2,

(f) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is attached to Het by a ring carbon atom,

(g) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of 3 to about 5 carbon atoms,

(h) aryl which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3 respectively,

(i) heteroaryl of 5 to 6 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3,

(j) aralkyl of about 7 to about 10 carbon atoms which is optionally mono-, di- or tri-substituted on the aryl ring with Y_1, Y_2 and/or Y_3;

(k) heteroaralkyl of 6 to 9 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted on the ring with Y_1, Y_2 and/or Y_3,

(l) aralkenyl of 8 carbon atoms which is optionally mono-, di- or tri- substituted on the aryl ring with Y_1, Y_2 and/or Y_3,

(m) heteroaralkenyl of 7 to 8 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $-S(O)_i-$ wherein i is independently 0, 1 or 2, and which is optionally mono-,

di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3,

(n) halogen,

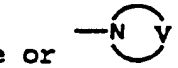
(o) difluoromethyl or perfluoroalkyl of 1 to 3 carbon atoms,

(p) perfluorophenyl,

(q) perfluoroaralkyl of 7 to about 9 carbon atoms, and

(r) alkoxy of 1 to about 10 carbon atoms;

(3) R_6 is selected from the group consisting of

(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, and halogen, wherein n is 0, 1 or 2, and R_1 is independently selected and as defined above, with the proviso that R_6 is not a camphor derivative or  heterocyclo group, and

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, $-OR_9$, $-NHR_9$, $-C(O)OH$, $-C(O)OR_9$, $-S(O)_2OH$ and $-S(O)_pR_9$ wherein R_9 is selected from alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 10 carbon atoms optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 12 carbon atoms optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, heteroaryl of 1 to about 9 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and heteroaralkyl of about 2 to about 10 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and

(4) R_7 is independently selected from the R_5 group of substituents, provided that R_7 is not halogen;

and pharmaceutically acceptable salts thereof.

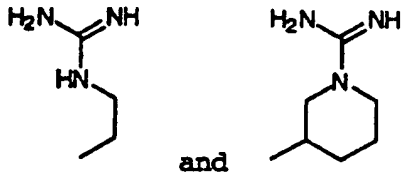
Preferred X groups include $-SO_2-$, $-NH-S(O)_2-$, and $-N(R')-S(O)_2-$. Especially preferred X groups include $-SO_2-$.

Preferred R_1 groups include alkyl, aralkyl, and aryl groups. Preferred R_1 aryl groups include substituted or unsubstituted phenyl and naphthyl. Preferred substitutions include, methyl, methoxy, fluoro, chloro, trifluoromethyl, and $-OCF_3$. Meta and ortho substitution is preferred.

Particularly preferred R_1 groups include aralkyl groups. Especially preferred R_1 groups include substituted or unsubstituted benzyl and naphthyl groups. Cyclohexyl and cyclohexylmethyl are other especially preferred R_1 groups.

A particularly preferred R_2 group is hydrogen.

Preferred R_3 groups include



and

Preferred R_4 groups include:

(i) hydrogen,

(ii) alkyl of 1 to 6 carbon atoms or alkyl of 1 to 6 carbon atoms substituted with Z_5, wherein Z_5 is selected from the group consisting of hydroxy, halogen, $-C(O)OH$, $-C(O)OR_8$, $-S(O)_3OH$ and $-S(O)_pR_8$ wherein R_8 is alkyl of 1 to about 6 carbon atoms, and p is 0, 1 or 2,

(iii) alkyl of 1 to 3 carbon atoms substituted with cyclic alkyl of 3 to 5 carbon atoms,

(iv) alkenyl of about 3 to about 6 carbon atoms,

(v) cycloalkyl of about 3 to about 5 carbon atoms,

(vi) heteroaryl of 5 atoms, and

(vii) heteroaralkyl of 6 atoms.

Preferred R_5 groups include hydrogen, halogen, alkyl of 1 to about 5 carbon atoms, trifluoromethyl, and alkoxy of 1 to 4 carbon atoms. Hydrogen is an especially preferred R_5 group.

Preferred R_6 groups include:

(i) hydrogen,

(ii) alkyl of 1 to about 12 carbon atoms or alkyl of 1 to 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, -OR_9, -NHR_9--C(O)OH, -C(O)OR_9, -$S(O)_2OH$ and -$S(O)_pR_9$, wherein R_9 is as defined above;

(iii) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 6 to about 8 carbon atoms;

(iv) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms or aryl of about 3 to about 10 carbon atoms;

(v) aralkyl or substituted aralkyl, as defined above;

(vi) heteroaralkyl or substituted aralkyl, as defined above;

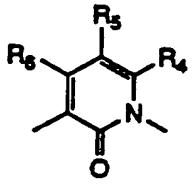
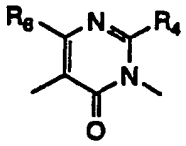
(vii) aralkenyl of about 8 to 15 carbon atoms which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, as defined above;

(viii) heteroaralkenyl or substituted heteroaralkenyl, as defined above.

More preferred R_6 groups, when R_4 and R_5 are hydrogen or methyl, are selected from the group consisting of aralkyl of about 8 to about 13 carbon atoms, and -O-aralkyl, -NH-aralkyl, and -$S(O)_p$-aralkyl of about 7 to about 12 carbon atoms. Preferred aryl portions of the aralkyl groups include unsubstituted and substituted phenyl or naphthyl. Preferred substitutions on the aryl ring include methyl, methoxy, fluoro, chloro and trifluoromethyl. Phenylethyl, phenylpropyl, hydrogen, cyclohexylethyl and cyclohexylpropyl are especially preferred R_6 groups.

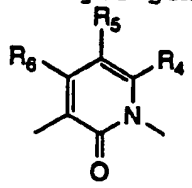
Preferred R_7 groups include hydrogen, methyl, difluoromethyl and trifluoromethyl. Hydrogen is an especially preferred R_7 group.

Pref rred Het groups include

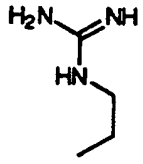
and

A particularly preferred Het, when R_5 and R_6 are independently selected to be hydrogen or methyl, is

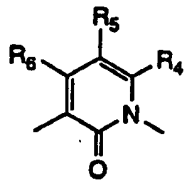


wherein R_4 is selected from the group consisting of hydrogen, methyl, ethyl, propenyl, allyl, propyl, isopropyl, butyl, R-sec-butyl, S-sec-butyl, isobutyl, 1-pentyl, R-2-pentyl, S-2-pentyl, 3-pentyl, S-1-(2-methyl)-butyl, R-2-(3-methyl)-butyl, 1-(3-methyl)-butyl, R-1-(2-methyl)-butyl, cyclopentyl, 2-pyrolyl, 3-pyrolyl, 1-hexyl, S-2-hexyl, R-2-hexyl, R-3-hexyl, and S-3-hexyl. A particularly preferred Het according to this aspect has hydrogen or methyl as R_4.

According to a particularly preferred aspect, provided are compounds of formula I wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aryl or aralkyl, R_3 is



and Het is



A very preferred aspect is directed to such compounds where R_1 is substituted or unsubstituted benzyl or phenyl.

Pr ferred compounds include 3-[(phenylsulfonyl)amino-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90, Compound B),

3-[(2-naphthylsulfonyl)amino]-2-oxo-1,2 dihydropyridyl-acetyl-L-argininal (Example 90),
3-[(1-naphthylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90),
3-(cyclohexylaminosulfonylamino-2-oxo-1,2-dihydropyridyl)-acetyl-L-argininal (Example 90),
3-(phenylaminosulfonylamino-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(phenoxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(cyclohexylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl-acetyl-L-argininal,
3-[(cyclohexylmethylsulfonyl)amino]-2-oxo-1,2 dihydro-pyridylacetyl-L-argininal (Example 121H),
3-[(phenethylsulfonyl)amino]-2-oxo-1,2-dihydro-pyridylacetyl-L-argininal (Example 121G),
3-[(2-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(3-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(4-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(2-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90),
3-[(3-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90),
3-[(4-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90),
3-[(2-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(3-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(4-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
3-[(2-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 90),
3-[(3-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal (Example 121L),

3-[(4-triflu romethylbenzylsulf nyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl] acetyl-L-argininal (Example 10),
[3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal (Example 19 and Example 113, Compound C),
5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinyl-acetyl-L-argininal (Example 29b),
2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal (Example 40 and 113, Compound D),
5-benzylsulfonylamino-uracilylacetyl-L-argininal,
5-benzylsulfonylamino-1-methyl-uracilylacetyl-L-argininal (Example 54 and Example 113, Compound E),
3-[(2-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl acetyl-L-argininal,
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl] acetyl-3-[3-piperidyl-(N-guanidino)]alaninal, and
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl] acetyl-D,L-3-amidinophenyl alaninal.

According to another aspect, the present invention is directed to salts of the compounds of formula (I). "Salt" includes within its definition, salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice, the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention. These salts include acid addition salts, for example, salts of hydrochloric acid, hydrobromic acid, acetic acid, benzene sulfonic acid and other suitable acid addition salts.

2. Preparation of Preferred Compounds

Figure 1 exemplifies a preferred reaction scheme for the synthesis of certain compounds of the present invention. N^g-nitro-L-argininal ethyl cyclol,

hydrochlorid salt 2 is c upled to the terminal carboxyl of the R_1 sulfonyl amino heterocycle 1 to give 3. Especially preferred coupling reagents are N-hydroxybenzotriazole in acetonitrile with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt. 3 is hydrogenated with hydrogen gas and palladium on carbon to remove the N^g-nitro group to give 4. 4 is then treated with strong acid and purified by HPLC with trifluoroacetic acid in the solvent to produce argininal 5.

The compounds of the present invention may be prepared by the preferred reaction schemes depicted in Figures 2 through 5. Examples 5 through 10 provide the details of the preferred reaction scheme of Figure 2, Examples 20 through 29 provide the details for the preferred reaction scheme of Figure 3, Examples 41 through 47 provide the details for the preferred reaction scheme of Figure 4, and Examples 55 through 63 provide the details for the preferred scheme of Figure 5.

In these reaction schemes, intermediates, which include 9, 18, 26, and 36 shown in Figures 2 through 5, respectively, are coupled to argininal or argininal mimic moieties to eventually give the compounds of the present invention. Examples 1 through 4 provide the details for the preparation of the precursor to the argininal moiety used in Figures 2 through 4. Examples 55 through 57 provide the details for the preparation of the argininal precursor used when hydrogenation sensitive groups exist. Examples 64 through 71 provide the details for the preparation of compounds of the present invention possessing a 3-[3-piperidyl-(N-guanidino)]alaninal in the P_1 position.

The preferred means of chemically coupling (as for example, 9 to 10 of Figure 2 or 18 to 19 of Figure 3) include formation of a peptide bond by using conventional coupling reagents known in the art. See Bodanszky, N., Peptide Chemistry, pp. 55-73, Springer-Verlag, New York (1988) and references cited therein. The chemical coupling may b either by means of one-step or two-step

coupling. In one-step coupling, the two coupling partners are coupled directly. Preferred coupling reagents for one-step coupling of the include DCC with HOBt, EDC with HOBt, HBTU or TBTU. In two-step coupling, an activated ester or anhydride of the C-terminal carboxy group of one coupling partner is formed prior to its coupling to the other coupling partner.

For example, as shown in Figure 2, the nitrogen of the pyridine ring of **6** is alkylated to give **7**. The nitro group is then reduced to the amine, which is then reacted with a sulfonyl chloride, depicted by R_1-S(O)$_2$-Cl, to give **8**. R_1 is as defined herein. The ethyl ester of **8** is removed by treatment with aqueous sodium hydroxide in methanol to give the carboxylic acid **9**. The acid of **9** is coupled to N^g-nitro-L-argininal ethyl cyclol HCl salt by carbodiimide coupling to give **10**. 6-Alkylated pyridyl compounds are made according to Examples 11 through 19. **10** is hydrogenated with hydrogen gas and palladium on carbon to remove the N^g-nitro group to give **11**. **11** is hydrolyzed in aqueous acid to give **12**.

Figure 3 provides a preferred reaction scheme for preparing pyrimidyl compounds of the present invention. Examples 21 through 29 describe this preparation. Pyrimidine **14** is alkylated with allyl bromide, and then the ester is hydrolyzed with sodium hydroxide in methanol to give the 1-allyl pyrimidone **15**. **15** is then treated with triethylamine and diphenylphosphoryl azide to form the acyl azide which undergoes the Curtius rearrangement. Reaction with t-butanol forms the BOC protected 5-aminopyrimidone **16**. Treatment with acid removes the BOC group. The amine is then reacted with an alkyl sulfonyl chloride to give **17**. **17** is oxidized in three steps to form **18**, which undergoes coupling as previously described.

Figure 9 provides an alternate preferred reaction scheme for preparing intermediate compound **18** of Figure 3. Synthesis of **18** by this alternate route is as described in Examples 102 to 107.

Figure 4 provides a preferred reaction scheme for preparing uracil compounds of the present invention. Examples 41 through 54 describe this preparation. As shown in Figure 4, 5-nitrouracil **22** is reacted with 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane to give the 5-nitrouracil bis(trimethylsilyl) ether, which is then reacted with bromomethylmethyl ether to give the methoxymethyl uracil **23**. This compound is then reacted with ethyl bromoacetate to give the ethyl uracilylacetate **24**. The nitro group is then reduced to the amine using hydrogen gas and palladium on carbon. The amine is then treated with 2,4,6-collidine and R_1SO_2Cl to give the amide **25**. The ethyl ester is converted to acid **26** by treatment with sodium hydroxide in methanol. The acid of **26** is coupled to N^g-nitro-L-argininal ethyl cyclol hydrochloride salt (prepared according to Examples 1 through 4). The adduct **27** is deprotected by treatment with hydrogen gas and palladium on carbon in an ethanol, acetic acid, and water mixture. **28** is hydrolized with 3N hydrochloric acid and then purified by HPLC with a solvent containing 0.1% trifluoroacetic acid to give argininal **29**.

Figure 10 provides an alternate preferred reaction scheme for preparing intermediate compound **26** of Figure 4. Synthesis of **26** by this alternate route is as described in Examples 108 to 111.

Figure 5 provides a preferred reaction scheme for preparing compounds of the invention possessing a hydrogenation sensitive moiety in the P_4 position. This method uses the di-N-t-butoxycarbonyl protecting group for the L-argininal moiety. This scheme has an alkenyl carbamate as the hydrogenation sensitive moiety. Examples 55 through 63 describe this preparation which uses hexafluorophosphoric acid to remove the BOC protecting groups. This general method can be used to prepare other hydrogenation sensitive compounds.

As described by Example 58, 3-nitro-2-hydroxypyridine **33** is treated with sodium hydride and then t-butyl bromoacetate to give **34**. The nitro group of **34** is

reduced to the amine by treatment with hydrogen gas and palladium on carbon. The amine is condensed with allyl chloroformate in the presence of sodium bicarbonate to give **35**. The t-butyl group of **35** is removed by trifluoroacetic acid to give **36**. Alpha-N-t-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonylarginine is dissolved in acetonitrile and treated with hydroxybenzotriazole and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl salt to form alpha-N-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-L-arginine lactam. The lactam **30** is opened by treatment with $LiAlH_4$ in tetrahydrofuran at -70°C to provide alpha-N-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-L-argininal **31**. This aldehyde is protected as the diethyl acetal by treatment with ethanol and HCl. The N-benzyloxycarbonyl protecting group is removed by treatment with hydrogen gas and palladium on carbon to give omega, omega'-di-N-t-butoxycarbonyl-L-argininal diethyl acetal, HCl salt **32**. This protected L-argininal moiety can then be coupled to a desired carboxylic acid, shown in the figure as **36**, by treatment with N-hydroxybenzotriazole and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl salt. The diethyl acetal and the di-BOC protecting groups are removed by treatment with hexafluorophosphoric acid in acetonitrile at 0°C. The reaction is quenched by adjusting to pH 4 with 2.5 M aqueous sodium acetate. Preparative HPLC using 0.1% CF_3COOH in 10-40% aqueous acetonitrile provides the trifluoroacetate salt of the desired substituted L-argininal compound **38**.

For preparation of certain compounds having hydrogenation sensitive substituent groups, it is preferred to avoid the use of hydrogen gas with palladium on carbon. Another preferred method for preparing compounds of the present invention containing hydrogenation sensitive groups such as alkenyl or aryl moieties substituted with halogen, cyano, nitro, or $-S-Z_1$, is to use boron tris(trifluoroacetate), $B(OCOCF_3)_3$, to cleave the N^g-nitro of the arginine group. The reagent is

prepared by the reaction of BBr_3 and CF_3COOH in dichloromethane at 0°C. The reagent is also commercially available. Generally, the N^g-nitro compound is treated with boron tris(trifluoroacetate) in trifluoroacetic acid at 0°C. *See, e.g.*, Fieser, M. and Fieser, L. F., Reagents for Organic Synthesis, p. 46, John Wiley & Sons, New York (1974); Pless, J., and Bauer, W. Angew. Chem., Internat. Ed., 12, 147 (1973).

In addition, another preferred reagent for selective nitro group cleavage is titanium trichloride. This reagent is commercially available. The Ng nitro compound is treated with titanium trichloride in aqueous methanol containing an ammonium acetate buffer followed by exposure of the reaction mixture to air or dimethyl sulfoxide. Freidinger, R.M., Hirschmann, R., and Veber, D.F., J. Org. Chem., 43, 4800 (1978).

Figure 7 illustrates a preferred reaction scheme for the preparation of compounds where X is a direct link. This figure is described by Examples 83 through 88.

As shown in Figure 7, the nitro group of pyridone **7** is reduced by treatment with hydrogen gas and palladium on carbon. The amine is then protected by the Boc group to form **39**. The Boc protected amine **39** is then treated with sodium hydride and alkylated with R_1 iodide, where R_1 is as defined herein. The ethyl ester is converted to acid **40** by sodium hydroxide. Acid **40** is then coupled to the compound of Example 4 by standard coupling techniques using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt, 1-hydroxybenzotriazole monohydrate, and N-methylmorpholine to give **41**. The N^g-nitro group is removed by catalytic hydrogenation with palladium on carbon to give **42**. The Boc protecting group is removed and the argininal is unmasked by treatment with HCl, followed by sodium acetate. HPLC purification with 0.1% trifluoroacetic acid gives the final product **43** in Figure 7.

Figure 8 provides an alternate reaction scheme for preparing the intermediate compound described in Example

16. Examples 91-96 describe this alternate synthetic route.

Figure 11 provides a preferred reaction scheme for the preparation of 6-substituted pyridones. Examples 97-101 describe this synthetic route.

Figure 12 provides a preferred reaction scheme for the preparation of 4-(hydroxyl substituted)alkyl or aralkyl pyridones. Examples 113-115 describe this synthetic route.

Figure 13 provides a reaction scheme for a preferred compound of the present invention, [3-(2-fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal, Examples 135 to 143 describe this synthetic route. An overall yield of 19% compound 83 was obtained.

Another aspect of the present invention is a method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the compound with a solution of a zinc salt and an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with an oxidizing agent, and

(c) recovering a 4-alkyl-3-nitro-2-xo-1,2-dihydropyridyl acetate product.

The disclosed method provides an efficient route to the synthesis of an intermediate used int he synthesis of compounds having a 6-membered heterocycle comprising an alkyl at the four position and a ntiro at the three position. Example 134 exemplifies several such compounds.

Zinc salts such as zinc chloride, zinc bromide, and zinc iodide can be used in step (a). Zinc chloride and zinc bromide are preferred, and zinc chloride is especially preferred.

An alkyl grignard results from reacting a magnesium metal with an alkyl or aryl bromide, chloride, or idodide. The magnesium inserts betwen the halogen and the carbon

bond to form the grignard. Acc rding t this aspect f the invention, the alkyl grignard in (a) is synthesized from a starting compound in the group defined by [R1]. A preferred alkyl grignard is 3-phenylpropyl magnesium bromide.

Step (a) can be performed by combining the compund and the zinc salt, follwed by addition of the alkyl grignard, or by combining the zinc salt and the grignard, followed by adition of the compound. Preferred is a method wherien the compound and the zinc salt are combined, followed by addition of the alkyl grignard.

The step (b) oxidation step can be performed using oxidizing agents including oxygen, catalytic reoxidation of palladium, dichlorodicyanoquinone in refluxing xylenes, and palladium acetate in warm THF. A preferred oxidizing agent is palladijm acetate in warm THF.

Examples 127-129 herein describe a preferred aspect of this method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the compound with zinc chloride and then adding an alkyl grignard under anhydrous coditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with palladium acetate in warm THF, and

(c) recovering a 4-alkyl-3-nitro-2-oxo-1,2-dihydropyridyl acetate product.

The compound of Example 129 is an intermediate in the synthesis of compounds such as those in Example 134.

In this preferred aspect the 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound is t-butyl[3-nitro-2-oxo-1,2-dihydropyridyl]acetate and the 4-alkyl-3-nitro-2-oxo-1,2-dihydropyridyl acetate product is t-butyl [3-nitro-2-oxo-4-(3-phenylpropyl)-1,2-dihydropyridyl]acetate.

A further aspect of the present invention is a method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the comp und with a solution of a zinc salt and an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with a reducing agent, and

(c) recovering a 4-alkyl-3-amino-2-oxo-piperidyl acetate product.

The disclosed method provides an efficient route to the synthesis of an intermediate used in the synthesis of copounds having a 6-membered heterocycle comprisng an alkyl at the four position and an amino at the three position.

Zinc salts such as zinc chloride, zinc bromide, and zinc iodide can be used in step (a). Zinc chloride and zinc bromide are preferred, and zinc chloride is especially preferred.

The alkyl grignard in (a) is synthesized fro a starting compound in the group defined by [R1]. A preferred alkyl grignard is 3-phenylpropyl magnesium bromide.

Step (a) can be performed by combining the compound and the zinc salt, followed by addition of the alkyl grignard, or by combining the zinc salt and the grignard, followed by addition of the compound. Preferred is a method wherein the compound and the zinc salt are combined, folled by addition of the alkyl grignard.

The step (b) reduction step can be performed with a reducing agent such as hydrogen, which is preferred.

On aspect of ths method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate copound at ring position 4 comprises

(a) combining the compound with zinc chloride and then adding an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with hydrogen, and

(c) recovering a 4-alkyl-3-amino-2- xo-piperidyl acetate product.

3. Selection of Preferred Compounds

The compounds of the present invention are screened for their ability to inhibit some or all of thrombin, factor Xa, plasmin, recombinant tissue plasminogen activator (rt-PA), activated protein C (aPC), chymotrypsin, and trypsin as set forth below. Certain of the preferred compounds are distinguished by their ability to inhibit thrombin, while not substantially inhibiting some or all of factor Xa, plasmin, t-PA, aPC, chymotrypsin, and trypsin. With respect to thrombin and the other enzymes and as used herein, the term "not substantially inhibiting" means that the IC_{50} (or K_i) for plasmin, t-PA, aPC, chymotrypsin, and trypsin for a given compound is greater than or equal to its IC_{50} (or K_i, respectively) for thrombin.

The compounds of the present invention are dissolved in buffer to give solutions containing concentrations such that assay concentrations range from 0 to 100 micromolar. In the assays for thrombin, factor Xa, plasmin, t-PA, aPC, chymotrypsin, and trypsin, a chromogenic synthetic substrate is added to a solution containing test compound and the enzyme of interest, and the residual catalytic activity of that enzyme is determined spectrophometrically. The IC_{50} of a compound of the present invention is determined from the rate of substrate turnover caused by the specific enzyme being measured. IC_{50} is that concentration of test compound giving 50% inhibition of the rate of substrate turnover. Likewise, the K_i of a compound of the present invention is determined from the rate of substrate turnover caused by the specific enzyme being measured at various enzyme concentrations. K_i is that concentration of test compound giving 50% inhibition of the rate of substrate turnover. Examples A and B provide an exemplar of the *in vitro*

assays used to select th compounds of the present invention.

Certain of the preferred compounds of the present invention have a K_i of about 0.001 to about 200 nM in the thrombin assay. Especially preferred compounds have a K_i of about 0.001 to about 50 nM. The more especially preferred compounds have a K_i of about 0.001 to about 10 nM.

Certain of the preferred compounds of the present invention have a IC_{50} for factor Xa, plasmin, t-PA, aPC, chymotrypsin, and trypsin which is at least 10 times greater than its IC_{50} for thrombin. Especially preferred compounds have an IC_{50} for factor Xa, plasmin, rt-PA, aPC, chymotrypsin, and trypsin which is about 20 to about 100,000 times greater than its IC_{50} for thrombin. More especially preferred compounds have an IC_{50} for factor Xa, plasmin, rt-PA, aPC, chymotrypsin, and trypsin which is about 100 to about 1,000,000 times greater than its IC_{50} for thrombin. In the event that a compound of the present invention has an IC_{50} with respect to factor Xa, plasmin, rt-PA, aPC, chymotrypsin, or trypsin which is greater than the highest concentration of compound tested, the IC_{50} is taken to be that highest concentration of compound.

Example B also provides a method for identifying and selecting compounds of the present invention that inhibit factor Xa, plasmin, t-PA, aPC, chymotrypsin and trypsin to a greater extent than they inhibit thrombin and, thus, have utility as inhibitors of those proteases.

4. Pharmaceutical Compositions

In another aspect, the present invention encompasses pharmaceutical compositions prepared for storage or administration which comprise a therapeutically effective amount of a compound of the present invention in a pharmaceutically acceptable carrier.

The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the

physical characteristics f the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

The therapeutically effective amount of the compound of the present invention can range broadly depending upon the desired affects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

The pharmaceutical compositions of the present invention may be formulated and used as tablets, capsules or elixers for oral administration; suppositories for rectal administration; sterile solutions and suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

When administration is to be parenteral, such as intravenous on a daily basis, injectable pharmaceutical compositions can be prepared in conventional forms, either

as liquid solutions or suspensions, solid f rms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxilliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

5. Utility and Methods

Compounds of the present invention when made and selected as disclosed are useful as potent inhibitors of thrombin *in vitro* and *in vivo*. As such, these compounds are useful as *in vitro* diagnostic reagents to prevent the clotting of blood and as *in vivo* pharmaceutical agents to prevent, inhibit and/or attenuate thrombosis in mammals suspected of having a condition characterized by abnormal thrombosis.

The compounds of the present invention are useful as *in vitro* diagnostic reagents for inhibiting clotting in blood drawing tubes. The use of stoppered test tubes having a vaccum therein as a means to draw blood obtained by venipuncture into the tube is well known in the medical arts. Kasten, B.L., "Specimen Collection", Laboratory Test Handbook, 2nd Edition, Lexi-Comp Inc., Cleveland pp. 16-17 (Edits. Jacobs, D.S. et al. 1990). Such vacuum tubes may be free of clot-inhibiting additives, in which case, they are useful for the isolation of mammalian serum from the blood. They may alternatively contain clot-inhibiting additives (such as heparin salts, EDTA salts, citrate salts or oxalate salts), in which case, they are useful for the isolation of mammalian plasma from the blood. The compounds of the present invention are potent inhibitors of thrombin, and as such, can be incorporated into blood coll ction tubes to prevent clotting of the mammalian blood drawn into them.

The compounds of the present invention are used alone, in combination with other compounds of the present invention, or in combination with other known inhibitors of clotting, in the blood collection tubes. The amount to be added to such tubes is that amount sufficient to inhibit the formation of a clot when mammalian blood is drawn into the tube. The addition of the compounds to such tubes may be accomplished by methods well known in the art, such as by introduction of a liquid composition thereof, as a solid composition thereof, or liquid composition which is lyophilized to a solid. The compounds of the present invention are added to blood collection tubes in such amounts that, when combined with 2 to 10 mL of mammalian blood, the concentration of such compounds will be sufficient to inhibit clot formation. Typically, the required concentration will be about 1 to 10,000 nM, with 10 to 1000 nM being preferred.

The compounds of the present invention are useful as a pharmaceutical agent for preventing, inhibiting and/or attenuating thrombosis in a mammal suspected of having a condition characterized by abnormal thrombosis.

Conditions characterized by abnormal thrombosis are well known in the medical arts and include those involving the arterial and venous vasculature of mammals. With respect to the coronary arterial vasculature, abnormal thrombosis (thrombus formation) characterizes the rupture of an established atherosclerotic plaque which is the major cause of acute myocardial infarction and unstable angina, as well as also characterizing the occlusive coronary thrombus formation resulting from either thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA). With respect to the venous vasculature, abnormal thrombosis characterizes the condition observed in patients undergoing major surgery in the lower extremities or the abdominal area who often suffer from thrombus formation in the venous vasculature resulting in reduced blood flow to the affected extremity and a predisposition to pulmonary embolism. Abnormal

thrombosis further characterizes disseminated intravascular coagulopathy which commonly occurs within both vascular systems during septic shock, certain viral infections and cancer, a condition wherein there is rapid consumption of coagulation factors and systemic coagulation which results in the formation of life-threatening thrombi occurring throughout the microvasculature leading to widespread organ failure.

The present invention includes methods for preventing a condition in a mammal suspected of having a condition characterized by abnormal thrombosis, comprising administering to said mammal a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

The compounds or pharmaceutical compositions of the present invention are administered *in vivo*, ordinarily in a mammal, preferably in a human. In employing them *in vivo*, the compounds or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Administration is preferably parenteral, such as intravenous on a daily basis. Alternatively, administration is preferably oral, such as by tablets capsules or elixers taken on a daily basis.

In practicing the methods of the present invention, the compounds or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic or *in vivo* diagnostic agents.

As is apparent to one skilled in the medical art, a "therapeutically effective amount" of the compounds or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treat d, the particular compounds employed, the particular mode of administration and the desired affects and the therapeutic indication. Because these factors and their

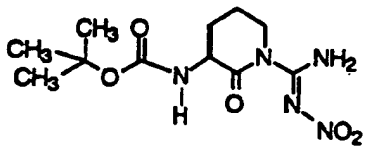
relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of preventing thrombosis, will be within the ambit of one skilled in these arts. Typically, administration of the compounds or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired effect of preventing *in vivo* thrombosis is achieved which would define a therapeutically effective amount. For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

To assist in understanding, the present invention will now be further illustrated by the following examples. These examples as they relate to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

Examples

Example 1

Preparation of N-alpha-t-butoxycarbonyl-N^{g}-nitro-L-arginine lactam

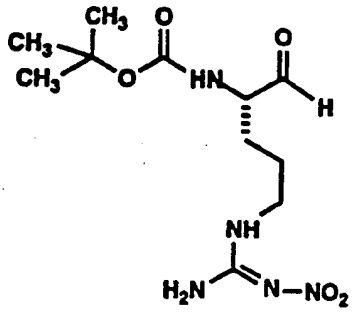


N-alpha-t-butoxycarbonyl-N^{g}-nitroarginine (2.00 g, 6.3 mmole) was dissolved in tetrahydrofuran (100 mL) by

heating the solution t 50°C. The solution was allowed to cool to room temperature. N-methyl piperidine (0.84 mL, 6.9 mmole) was added, and the solution was cooled in an ice bath. Isobutylchloroformate (0.83 mL, 6.3 mmole) was added, and the reaction mixture was stirred at 0°C for 6 hours. The reaction mixture was stirred for 18 hours while the ice in the Dewar was allowed to melt overnight. The solvent was removed under vacuum. The crude product was dissolved in 20% ethyl acetate/dichloromethane (10 mL), and was purified by flash chromatography through a 3x5 cm column of silica gel using 20% ethyl acetate/dichloromethane as eluent. 125 mL of eluent was collected. The solvent was removed under vacuum to afford 1.39 g (74% crude yield) of the title compound as a white foam. R_f = 0.44 (silica gel, 5% isopropanol in dichloromethane). Isobutanol was present as an impurity. This compound may be further purified by recrystallization from dichloromethane/hexanes or ethanol/water.

Example 2

Preparation of N-alpha-t-butoxycarbonyl-N^g-nitro-L-argininal



(a) Procedure 1

To a stirred solution of $LiAlH_4$ in tetrahydrofuran (3.8 mL of a 1.0M solution, 3.8 mmole), cooled in an ice bath, was added dropwise ethyl acetate (0.43 mL, 3.8 mmole) in tetrahydrofuran (5 mL). The solution was stirred for 30 minutes at 0°C to preform $LiAlH_2(OEt)_2$.

The solution of this $LiAlH_2(OEt)_2$ was added dropwise

to a stirred solution of compound of Example 1 (0.92 g, 3.1 mmole) in tetrahydrofuran (5 mL). After 30 minutes, the reaction is quenched with 1.0N HCl/tetrahydrofuran (2 mL of a 1:1 mixture). 1.0N HCl (20 mL) was added, and the solution was extracted three times with ethyl acetate (20 mL each). The combined organic layers were washed with water (5mL), saturated sodium bicarbonate (5 mL) and twice with brine(5 mL each), dried over anhydrous magnesium sulfate, filtered and the solvent was removed under vacuum to give 0.94 g (100% yield) of the title compound as an off-white solid.

(b) Procedure 2

Alternatively, the title compound was made by the procedures which follow.

A 12 liter four-necked round bottom flask equipped with an overhead stirring apparatus was flame dried under a strong stream of nitrogen. After the flask had cooled, 120.0 g of N-alpha-t-butoxycarbonyl-N^{g}-nitro-L-arginine (376 mmole, 1 equivalent) was added under a blanket of nitrogen followed by the addition of 6 liters of anhydrous tetrahydrofuran (Aldrich sure-seal) via canula. The flask was then fitted with a thermometer and the resulting suspension was warmed to 50°C with a heat gun while stirring. The reaction mixture was cooled to 5°C with an ice bath and further cooled to -5°C with an ice/acetone bath.

During the time it took for this solution to reach -5°C, 36.66 g of N-methyl-O-methylhydroxyamine hydrochloride (376 mmole, 1.0 equivalent) was weighed out in a 500 mL flask and suspended in 300 mL of dichloromethane. This suspension was sparged with nitrogen for 5 minutes, cooled to 0°C and 46 mL of N-methylpiperidine (1.0 equivalent) was added via syringe under nitrogen. The mixture was sonicated briefly to insure complete dissolution/free base f rmation and recooled to 0 C in an ice bath while still under nitrogen. The resulting solution of free base was used later.

When the above arginine solution had reached -5°C, 45 mL of N-methylpiperidine was added via syringe followed 5 minutes later by the addition of 46 mL of isobutyl chloroformate (0.95 equivalent) via syringe. The resulting solution was stirred for 15 minutes at -5°C. After this time, the free base solution of N-methyl-O-methyl hydroxylamine generated above was added via canula over about 15 minutes. Stirring was continued at -5°C for another 1.5 hours at which time thin layer chromatography (silica gel, 1:10:90 acetic acid/methanol/dichloromethane) indicated that the reaction was complete. The reaction mixture was filtered while still cold, the salts washed with 400 mL of cold tetrahydrofuran and the filtrate concentrated under vacuum on a rotary evaporator to yield a yellow foam.

The crude intermediate was taken up in 300 mL of dichloromethane and applied to a column of silica gel (70 - 230 mesh, 7 x 50 cm). The column was first eluted with 2 liters of dichloromethane followed by 2 liters of 2% methanol in dichloromethane. This was followed by elution with 5% methanol in dichloromethane until all of the product had been eluted (the eluant was checked for UV activity and five one-liter fractions were collected once this UV activity was apparent). Fractions containing pure product were pooled and concentrated under vacuum and pumped on overnight to yield 120.1 g (88% yield) of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine-(N-methyl, N-methoxyamide) as light yellow foam. This foam was taken up in 300 mL of dichloromethane, 300 mL of toluene, and the volatiles were once again removed under vacuum to remove any residual water or methanol.

120.1 g of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine-(N-methyl, N-methoxyamide) (331.4 mmole) was taken up in 2.8 liters of dry (Aldrich sure-seal) tetrahydrofuran and transferred to a dry 5 liter 4-necked round bottom flask equipped with a mechanical stirrer and a low temperature thermometer. The solution was cooled to -70°C with a dry ice/acetone bath and 300 mL of 1M $LiAlH_4$

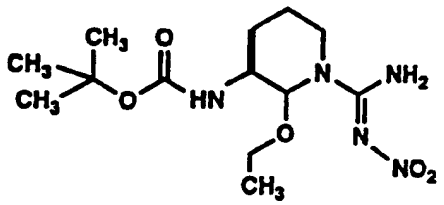
in tetrahydrofuran was added by canula transfer directly from 100 mL Aldrich sure-seal bottles. An additional 50 mL of 1M $LiAlH_4$ in tetrahydrofuran was added via syringe (total 331 mL). During the additions, the reaction temperature was kept below -60°C. The reaction was stirred for 0.5 hours at -70°C, the cooling bath removed, and the reaction was slowly allowed to warm to 0°C (about 2.5 hours). Between -30°C and -20°C a thick slurry resulted. When the reaction mixture obtained 0°C, a small aliquot was removed and partitioned between ethyl acetate/2M potassium bisulfate. The organic layer was analyzed by thin layer chromatography (silica gel, ethyl acetate).

When the reaction was judged to be complete, it was cooled to -70°C and 503 mL of 2M potassium bisulfate was added via dropping funnel at a slow enough rate to keep the reaction temperature below -30°C. The cooling bath was removed and the reaction mixture was allowed to come to 0°C over the course of 2 hours at which time a white precipitate was filtered off. The solids were washed with 500 mL of cold tetrahydrofuran. The filtrate was concentrated under vacuum on a rotary evaporator until most of the tetrahydrofuran was removed and the remaining white sludge was mostly aqueous. The crude product was taken up in 1.5 liters of ethyl acetate and washed with 0.2 M HCl (2 x 200 mL). The HCl extracts were back-extracted with 400 mL of ethyl acetate and the organics were combined and extracted with saturated sodium bicarbonate (2 x 200 mL). The bicarbonate extracts were also back-extracted with 400 ml of ethyl acetate. The organics were then combined and washed with brine (200 mL) followed by drying over anhydrous sodium sulfate. The solution was filtered, concentrated under vacuum on a rotary evaporator and pumped on overnight to yield a white solid (89.0 g) of crude title compound. This was chromatographed on silica gel and eluted with a gradient of 0 to 10% methanol in dichloromethane. The pure fractions were combined and evaporated to yield the title

compound as a white solid (75 g, 74%).

Example 3

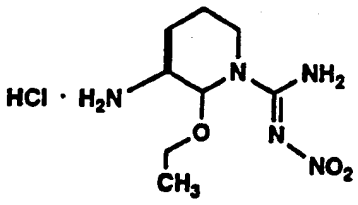
Preparation of N-alpha-t-butoxycarbonyl-N^{g}-nitro-L-argininal ethyl cyclol



The compound of Example 2 (41.60 g, 0.137 mole) was dissolved in ethanol (200 mL) and concentrated HCl (1 mL) was added. After the reaction was complete by TLC (silica gel, 10% methanol in dichloromethane), the solvent was removed under vacuum. The crude product was purified by flash chromatography through a column of silica gel (230-400 mesh) using 0 to 10% ethyl acetate/dichloromethane as eluent. The combined fractions yielded 36.88 g (81%) of the title compound as pale yellow foam. R_f = 0.62 (silica gel, 5% methanol in dichloromethane).

Example 4

Preparation of N^{g}-nitro-L-argininal ethyl cyclol, hydrochloride salt

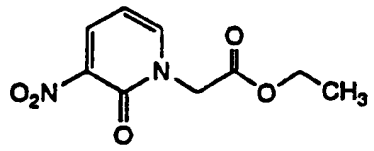


To a solution of the compound of Example 3 (35 g) in 500 mL of absolute ethanol at 0°C was added slowly 500 mL of absolut ethanol saturated with HCl(g). This mixture was allowed to warm to 25°C and checked by thin-layer chromatography. The appearance of a very polar product was the desired compound. Most of the HCl was removed with

a stream f dry nitrogen and the resulting organic solvent was removed under vacuum. The resulting 33 g of the title compound as a yellow-white solid was used without further purification.

Example 5

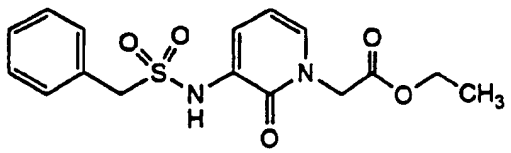
Preparation of ethyl (3-nitro-2-oxo-1,2-dihydropyridyl)acetate



Sodium hydride (4.08 g of a 60% dispersion in mineral oil, 0.10 mole) was washed with hexanes three times (10 mL each) and suspended in dimethylformamide (50 mL). The stirred suspension was cooled in an ice bath, then 3-nitro-2-hydroxypyridine (13.0 g, 0.093 mole) was added in small portions over a 45-minute period. After the addition was complete, the reaction was stirred at 0°C for 10 minutes, then room temperature for 30 minutes. The reaction mixture was recooled in an ice bath. Ethyl bromoacetate (0.75 mL, 0.097 mole) was added. The reaction was stirred at 0°C for 1 hour, then 1.5 hours at room temperature. The reaction mixture was partitioned between ethyl acetate (200 mL) and water (200 mL). The aqueous layer was extracted with ethyl acetate (3x200 mL). The combined organic extracts were washed with water (4x100 mL), brine, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was chromatographed through silica gel using 0 to 20% ethyl acetate/dichloromethane as eluent to afford 15.7 g (75% yield) of the title compound as yellow solid. R_f = 0.30 (silica gel, 20% ethyl acetate in dichloromethane).

Example 6

Preparation of ethyl (3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl)acetate

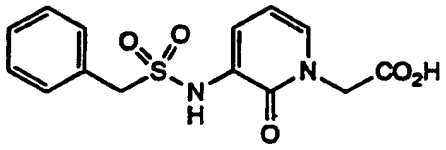


A stirred solution of the compound of Example 5 (44.5 g, 0.197 mole) in ethanol (200 mL) was hydrogenated over 10% Pd/C (2.25 g) for 16 hours under balloon pressure. Celite was added, and the reaction mixture was filtered through a pad of celite in a 600 mL fritted funnel (5 cm depth), using ethyl acetate to wash. The solvent was removed under vacuum, diluted with ethyl acetate (200 mL) and toluene (200 mL), and the solvent was removed under vacuum to give crude ethyl (3-amino-2-oxo-1,2-dihydropyridyl)acetate (40.0 g, 0.204 mole) in quantitative yield.

A stirred solution of ethyl (3-amino-2-oxo-1,2-dihydropyridyl)acetate (40.0 g, 0.204 mole) and 2,4,6-collidine (54 mL, 0.408 mole) in tetrahydrofuran (200 mL) was cooled in an ice bath. A solution of benzylsulfonyl chloride (38.9 g, 0.204 mmole) in tetrahydrofuran (200 mL) was added over a 50-minute period. After addition was complete, the solution was stirred for 30 minutes at 0°C. The reaction mixture was diluted with ethyl acetate (1.2 L), washed with 1.0N HCl (until aqueous layer is pH 1), water (50 mL), saturated sodium bicarbonate (100 mL), and brine (2x50 mL). The organic layer was dried over anhydrous magnesium sulfate, and the solvent was removed. The residue was recrystallized from chloroform. 39 g of the title compound was isolated. To the mother liquor was added silica gel. The solution was swirled, then filtered through a sintered glass funnel, washing with 50% ethyl acetate in dichloromethane. The solvent was removed from the filtrate, and the residue was recrystallized from chloroform. An additional 13 g of the title compound was isolated to afford a total of 52.00 g (75% yield) of the title compound as a tan solid. R_f = 0.32 (silica gel, 20% ethyl acetate in dichloromethane); m.p. 48-49°C.

Example 7

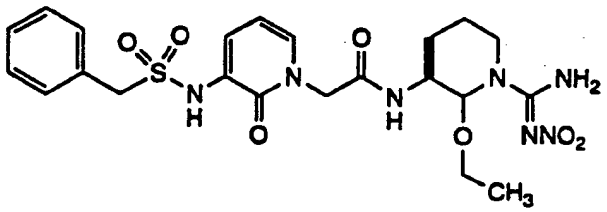
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetic acid



To a cooled (0°C) suspension of the compound of Example 6 (50.89 g, 0.145 mole) in methanol (500 mL) was added 1.0N NaOH (327 mL) over a period of 10 minutes. After the addition was complete, the solution was allowed to warm to room temperature over a period of 1.5 hours. The solution became homogeneous upon addition of NaOH. A precipitate formed during the reaction. The solvent was reduced under vacuum, the residue diluted with water (400 mL), and washed with ethyl acetate (2x150 mL). The aqueous layer was acidified with 2.0N HCl to pH 1, and extracted with ethyl acetate (3x200 mL). The combined organic extracts were washed with water, then brine (twice). The product crystallized. The 2 combined crops yielded 44.54 g (95%) of the title compound as off-white crystals. R_f = 0.17 (silica gel, 1% acetic acid, 10% methanol in dichloromethane); m.p. 186-187°C.

Example 8

Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-N^g-nitro-L-argininal ethyl cyclol

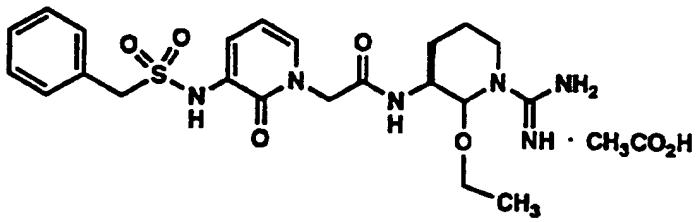


To a stirred suspension of the compound of Example 7 (23.3 g, 77 mmole), the compound of Example 4 (N^g-nitro-L-argininal ethyl cyclol, hydrochloride salt, 24.76 g, 92 mmole), and N-hydroxybenzotriazole (11.79 g, 77 mmole) in acetonitrile (400 mL) cooled to 0°C was added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (EDC, 17.76 g, 92 mmole). After 30 minutes, the solution

was almost homogeneous. N-m thylmorph line (25.4 mL, 231 mmole) was added dropwise. After the addition was complete, the reaction was stirred at room temperature for 3 hours. The solvent was reduced under vacuum, and the resulting residue was dissolved in dichloromethane (600 mL), washed with 50 mL each of 2.0N HCl (to pH 1), water, saturated sodium bicarbonate and brine. The extract was dried over anhydrous magnesium sulfate, and the solvent was removed under vacuum. The crude product was purified in three batches using a 600 mL fritted funnel as a column through silica gel (7 cm depth) to yield (29.40 g, 74%) of the title compound. Analytical HPLC gave t_R = 12.8 minutes (20-60% CH_3CN, 25 mm Vydac C-18 column). R_f = 0.28 (silica gel, 5% ethanol in dichloromethane).

Example 9

Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ethyl cyclol, acetate salt

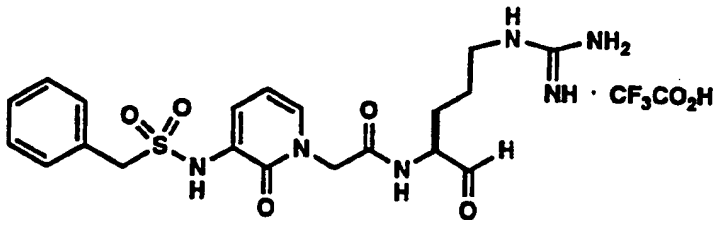


The compound of Example 8 (5.60 g, 11 mmole) in ethanol/acetic acid/water (4:1:1, 60 mL) was hydrogenated over 10% palladium on carbon (1.80 g) for 4 hours at 20 psi. Celite was added, and the solution was filtered through a 0.2 micron filter, washing the solid with ethanol/acetic acid/water (4:1:1, 60 mL). To the filtrate was added 10% palladium on carbon (1.80 g), and the solution was hydrogenated at 20 to 25 psi for 40 hours. Celite was added, and the solution was filtered through a 0.2 micron filter, washing the solid with wat r (200 mL). The solvent was reduced to a volume of 200 mL under reduced pressure, then washed with ethyl acetate (50 mL).

Th solvent from the aqueous layer was reduc d to remove the volatiles, then the aqueous layer was lyophilized to yield 4.88 g (85% yield) of the title compound. Analytical HPLC gave t_R = 9.5 minutes (20-60% CH_3CN, 25 mm Vydac C-18 column).

Example 10

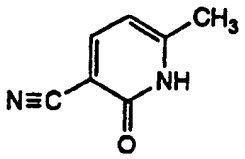
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, trifluoroacetate salt



The compound of Example 9 (4.88g, 9.2 mmole) was suspended in 3.0 N HCl (100 mL). After stirring for 3 h, the reaction mixture was quenched with 2.5 M aqueous sodium acetate to pH 3.5 to 4, then filtered through a 2 micron filter. The filtrate was purified in two batches by preparative HPLC (Waters PrepPak cartridge, Delta-Pak C18, 300 angstrom column, 0 to 40% acetonitrile/water containing 0.1% trifluoroacetic acid). The clean fractions were combined to give 2.05 g (40% yield) of the title compound. Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 463.

Example 11

Preparation of 6-methylpyrid-2-one-3-carbonitrile

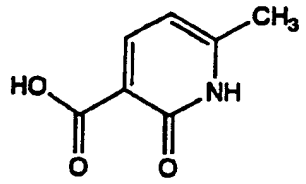


To stirred a mixture of sodium methoxide (46.5 g, 860 mmole) in diethyl ether (950 mL), cooled in an ice

bath, was added a mixture of acetone (46.5 g, 800 mmole) and ethyl formate (59.6 g, 800 mmole) dropwise over 1 hour. When addition was complete, the cooling bath was removed and the mixture was warmed to room temperature over a 1 hour period. The volatile materials were distilled, keeping the oil bath at no more than 60°C. To the solid residue was added cyanoacetamide (67 g, 800 mmole) in water (400 mL) and piperidine acetate (140 mmole, prepared by adding piperidine to a solution of 8.0 mL of acetic acid in 20 mL water until the solution was greater than pH 7). The flask was fitted with a reflux condenser, and the mixture was heated for 2 hours under reflux. The mixture was cooled to room temperature and acidified to pH 5 with acetic acid. After standing overnight at room temperature, the mixture was cooled in an ice bath for 45 minutes. The yellow solid product was filtered, washed with ice water four times and dried under vacuum at 80°C overnight. Crystallization from 50% (v/v) ethanol afforded the title compound as a yellow solid (52.6 g, 49% yield). R_f=0.29 (silica gel, 95:5 chloroform:methanol). Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 134.

Example 12

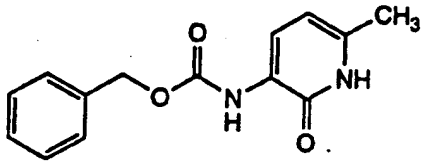
Preparation of 6-methylpyrid-2-one-3-carboxylic acid



A suspension of the compound of example 11 (16.9 g) in 20% NaOH (w/w, 63 mL) was heated at 140 to 145°C overnight in a sealed bomb. The cooled reaction mixture was acidified to about pH 8 with concentrated hydrochloric acid and extracted with dichloromethane (three times). The aqueous phase was acidified, precipitating a yellow solid which was filtered, washed with water, and dried overnight in a vacuum oven at approximately 80°C. The dried title compound (15.68 g, 81% yield) required no further purification. Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 153.

Example 13

Preparation of 3-benzyloxycarbonylamino-6-methylpyrid-2-one

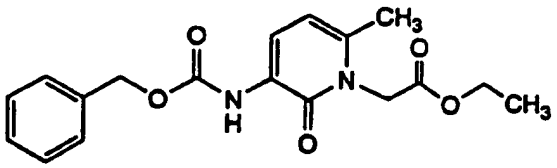


To the compound of Example 12 (11.8 g, 0.077 mole), suspended in dioxane (260 mL), triethylamine (11.3 mL, 0.081 mole) is added dropwise rapidly with stirring followed by diphenylphosphoryl azide (16.7 mL, 0.077 mole). The suspension is heated under reflux for 4 hours using a preheated 120°C oil bath. Benzyl alcohol (24.1

mL, 0.23 mole) is then added and the mixture was stirred under reflux overnight. The reaction mixture is cooled and evaporated. The residue is suspended in water (600 mL) and filtered. The filter cake is washed with 10% HCl twice, saturated sodium bicarbonate and brine. The crude product is chromatographed using 20 to 30% ethyl acetate/chloroform to give the title compound.

Example 14

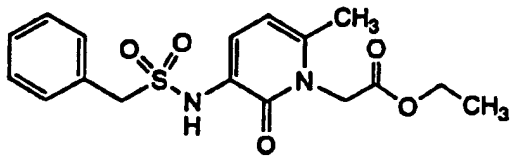
Preparation of ethyl (3-benzyloxycarbonylamino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetate



The compound of Example 13 (1.80 g, 7.0 mmole) was added to a stirred suspension of sodium hydride (0.33 g, 8.4 mmole) in dry dimethylformamide (50 mL). After 45 minutes, ethyl iodoacetate (1.43 g, 6.7 mmole) was added, and the mixture was stirred overnight, diluted with 10% hydrochloric acid (300 mL) and extracted with ethyl acetate (3x150 mL). The organic layer was washed with brine (twice), dried and evaporated. The resulting yellow, waxy solid was chromatographed, eluting with 3% ethyl acetate in dichloromethane, to give the title compound (1.28 g, 53% yield). R_f = 0.52 (silica gel, 5:95 methanol:dichloromethane). Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 344.

Example 15

Preparation of ethyl [3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetate

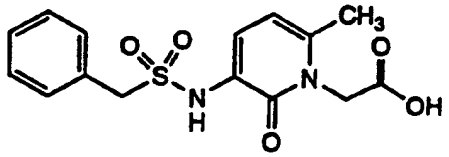


A stirred solution of compound of Example 14 (2.50 g) in ethanol (25 mL) is hydrogenated over 10% Pd/C (0.25 g) for 5 hours under balloon pressure. Celite is added, and the reaction mixture is filtered through a pad of celite, using ethyl acetate to wash. The solvent is removed under vacuum, diluted with ethyl acetate (20 mL) and toluene (20 mL), and the solvent is removed under vacuum to give crude ethyl (3-amino-6-methyl-2-oxo-1,2-dihydropyridyl)acetate.

A stirred solution of ethyl (3-amino-6-methyl-2-oxo-1,2-dihydropyridyl)acetate (0.55 g, 2.6 mmole) and 2,4,6-collidine (1.2 mL, 5.2 mmole) in tetrahydrofuran (10 mL) is cooled in an ice bath. A solution of benzylsulfonyl chloride (0.50 g, 2.6 mmole) in tetrahydrofuran (10 mL) is added over a 15 minute period. After addition is complete, the solution is stirred for 30 minutes at 0°C. The reaction mixture is diluted with ethyl acetate (100 mL), washed with 1.0N HCl (until aqueous layer is pH 1), water (10 mL), saturated sodium bicarbonate (10 mL), and brine (2x10 mL). The organic layer is dried over anhydrous magnesium sulfate, and the solvent is removed. The residue is chromatographed through silica gel using 0:100-20:80 ethyl acetate:dichloromethane as eluent to afford the title compound.

Example 16

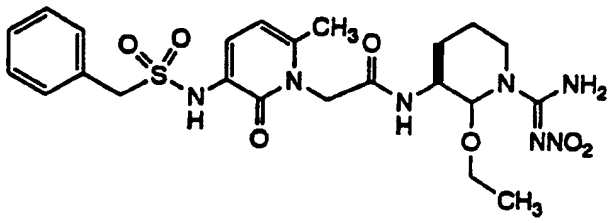
Preparation of [3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetic acid



Using similar procedures to that described above in Example 7, the title compound is prepared from the compound of Example 15. An alternative method of preparing the title compound is described in Examples 91 to 96.

Example 17

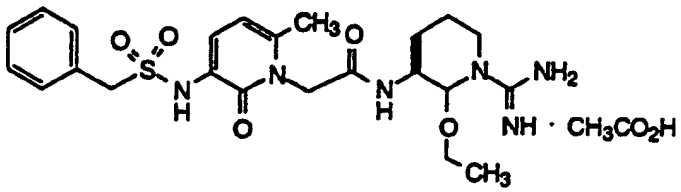
Preparation of [3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetyl-NG-nitro-L-argininal ethyl cyclol



Using similar procedures to that described above in Example 8, the title compound is prepared from the compound of Example 16.

Example 18

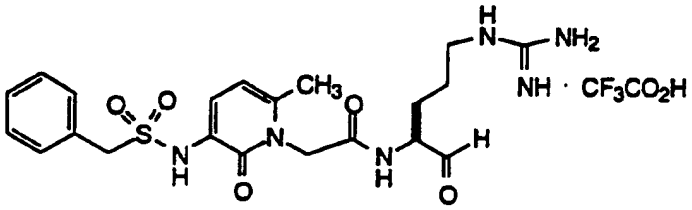
Preparation of [3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ethyl cyclol, acetate salt



Using similar procedures to that described above in Example 9, the title compound is prepared from the compound of Example 17.

Example 19

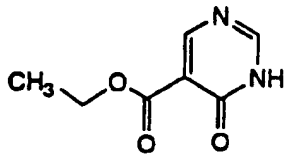
Preparation of [3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, trifluoroacetate salt



Using similar procedures to that described above in Example 10, the title compound is prepared from the compound of Example 18. An alternative method of preparing the title compound is described in Example 113 (Compound C).

Example 20

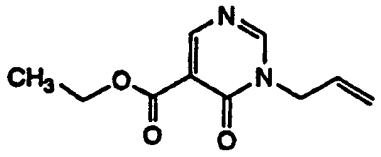
Preparation of ethyl pyrimidin-6(1H)-one-5-carboxylate



Diethyl ethoxymethylenemalonate (10.1 mL, 50 mmole) and formamidine acetate (10.4 g, 100 mmole) were refluxed in ethanol (10 mL) for 24 hours. The reaction mixture was allowed to cool to room temperature overnight, and suspended in ethyl acetate (30 mL) and 1.0N HCl (20 mL). The suspension was filtered, and the filter cake was washed with 1.0N HCl, followed by water, then ethyl acetate, and air dried affording the title compound as an tan solid (3.33 g, 40% yield). R_f = 0.21 (silica gel, 10% methanol in dichloromethane); m.p. 187-188°C.

Example 21

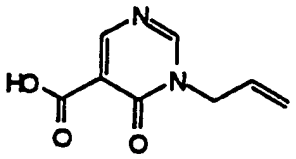
Preparation of ethyl 1-allyl-pyrimidin-6(1H)-one-5-carboxylate



The compound of Example 20 (4.3 g, 26 mmole) is added to a stirred suspension of sodium hydride (1.13 g, 28 mmole) in dry dimethylformamide (50 mL). After 45 minutes, allyl bromide (2.21 mL, 26 mmole) is added, and the mixture is stirred overnight, diluted with 10% hydrochloric acid (300 mL) and extracted with ethyl acetate (3x150 mL). The organic layer is washed with brine (twice), dried and evaporated. The residue is chromatographed through silica gel using 0-10% isopropanol/dichloromethane as eluent. The title compound is isolated.

Example 22

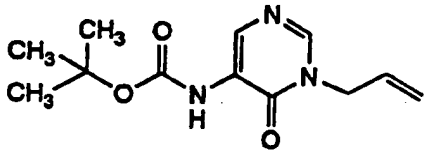
Preparation of 1-allyl-pyrimidin-6(1H)-one-5-carboxylic acid



To the compound of Example 21 (5.00 g, 0.024 mole), suspended in methanol (25 mL) and cooled in an ice bath, 1.0N NaOH (29 mL, 0.029 mole) is added dropwise rapidly with stirring. After 16 hours, the solvent is reduced under vacuum, residue diluted with water (50 mL), and washed with ethyl acetate (2x15 mL). The aqueous layer is acidified with 2.0N HCl to pH 1, extracted with ethyl acetate (3x50 mL). The combined organic extracts are washed with water, then brine (twice). The solvent is removed *in vacuo* to afford the title compound.

Example 23

Preparation of 1-allyl-5-t-butyloxycarbonylamino-6-oxo-1-pyrimidi-6(1H)-one

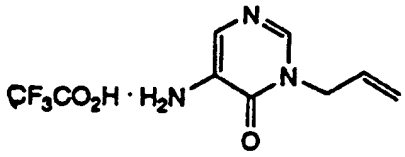


To the compound of Example 22 (10.0 g, 0.056 mole), suspended in dioxane (260 mL), triethylamine (8.1 mL, 0.058 mole) is added dropwise rapidly with stirring followed by diphenylphosphoryl azide (12.0 mL, 0.077 mole). The suspension is heated under reflux for 4 hours using a preheated 120°C oil bath. t-Butanol (12.3 g, 0.17 mole) is then added and the mixture is stirred under reflux overnight. The reaction mixture is cooled and evaporated. The residue is suspended in water (600 mL) and filtered. The filter cake is washed with 1.0N HCl

twice, saturated sodium bicarbonate and brine. The crude product is chromatographed using 0 to 50% ethyl acetate/dichloromethane to give the title compound.

Example 24

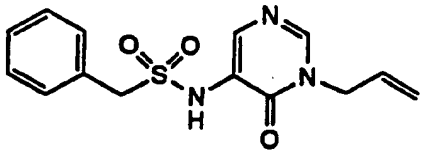
Preparation of 1-allyl-5-amino-6-oxo-1-pyrimidi-6(1H)-one, trifluoroacetate salt



The compound of Example 23 (5.00 g) is treated with 50% trifluoroacetic acid in dichloromethane (50 mL) for 35 minutes. The solution is added dropwise to diethyl ether (500 mL), while swirling. The precipitate is filtered, washing with diethyl ether. The powder is dried under vacuum to yield the title compound.

Example 25

Preparation of 1-allyl-5-benzylsulfonylamino-6-oxo-1-pyrimidi-6(1H)-one



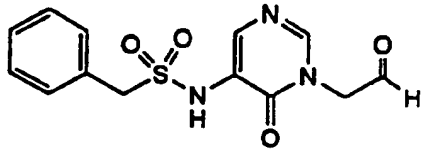
The free base of the compound of Example 24 is generated by dissolving the compound of Example 24 (5.00 g, 18.9 mmole) in 1M potassium carbonate. The free base is then extracted into dichloromethane, which is dried and evaporated to give 1-allyl-5-amino-6-oxo-1-pyrimidi-6(1H)-one.

A stirred solution of 1-allyl-5-amino-6-oxo-1-pyrimidi-6(1H)-one and 2,4,6-collidine (8.3 mL, 38 mmole) in tetrahydrofuran (25 mL) is cooled in an ice bath. A

solution of benzylsulfonyl chloride (3.59 g, 18.9 mmole) in tetrahydrofuran (25 mL) is added over a 15-minute period. After addition is complete, the solution is stirred for 1 hour at 0°C. The reaction mixture is diluted with ethyl acetate (200 mL), washed with 1.0N HCl (until aqueous layer is pH 1), water (25 mL), saturated sodium bicarbonate (25 mL), and brine (2x25 mL). The organic layer is dried over anhydrous magnesium sulfate and the solvent is removed *in vacuo* to give the title compound.

Example 26

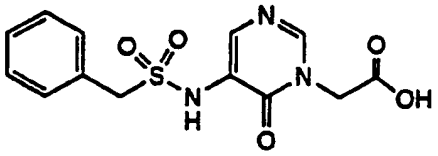
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetaldehyde



To a solution of the compound of Example 25 (5.00 g, 16.4 mmole) in tetrahydrofuran (50 mL) and water (7 mL) is added N-methylmorpholine-N-oxide (3.20 g, 16.4 mmole) and osmium tetroxide (1.0 mL of a 4% solution in water, 0.16 mmole). After the reaction mixture is stirred for 18 hours, N-methylmorpholine-N-oxide (0.47 g, 2.8 mmole) is added. After stirring the reaction mixture for 4 hours, sodium thiosulfate, (2.5 mL of a saturated aqueous solution) and diatomaceous earth (7 g) are added, and the mixture is stirred for 30 minutes. The mixture is filtered and evaporated under vacuum to give an oil. This oil is dissolved in ethanol (60 mL) and a solution of sodium periodate (7.00 g, 33 mmole) in water (10 mL) is added. The residue is dissolved in ethyl acetate and the solution is washed with water, dried, and evaporated to afford th title compound.

Example 27

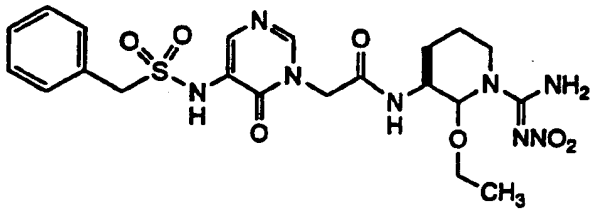
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetic acid



To the compound of Example 26 (5.00 g, 15.6 mmole) in t-butanol (40 mL) and 2-methyl-2-butene (33 mL) is added a solution of sodium chlorite (13 g, 14 mmole) and sodium dihydrogen phosphate monohydrate (15.1 g, 109 mmole) in water (40 mL). The reaction mixture is stirred for 3 hours, then concentrated under reduced pressure. The residue is diluted with ethyl acetate and extracted with 1.0N sodium hydroxide. The aqueous layer is acidified to pH 1 with 1.0N hydrochloric acid, then extracted with dichloromethane twice. The organic extracts are dried and evaporated to give the title compound.

Example 28

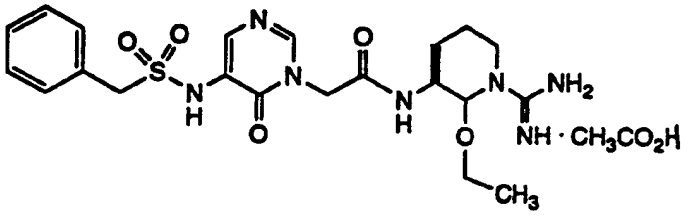
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-N^g-nitro-L-argininal ethyl cyclol



Using similar procedures to that described above in Example 8, the title compound is prepared from the compound of Example 27.

Example 29a

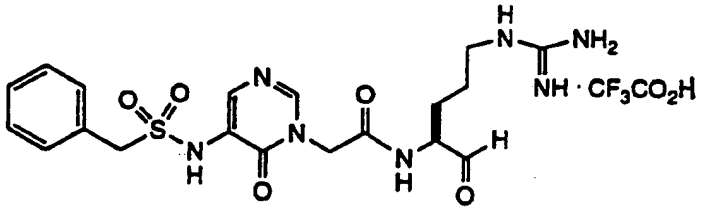
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal ethyl cyclol, acetate salt



Using similar procedures to that described above in Example 9, the title compound is prepared from the compound of Example 28.

Example 29b

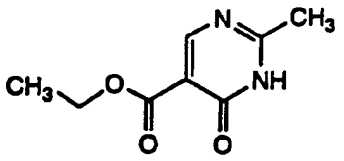
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal, trifluoroacetate salt



Using similar procedures to that described above in Example 10, the title compound is prepared from the compound of Example 29a.

Example 30

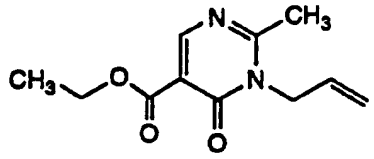
Ethyl 2-methyl-pyrimidin-6(1H)-one-5-carboxylate



Acetamidine hydrochloride (37.16 g, 0.39 mole) was stirred in sodium ethoxide in ethanol (73 mL of a 21% solution, 0.20 mole) for 5 minutes. Diethyl ethoxymethylenemalonate (31.5 mL, 0.15 mole) was added, and the reaction mixture was refluxed for 5 hours. The reaction mixture was allowed to cool to room temperature overnight, and diluted with dichloromethane (100 mL). The solution was filtered, washing the solid cake with dichloromethane. The filtrate was concentrated at reduced pressure. The residue was dissolved in dichloromethane (150 mL) and 2.0N HCl (30 mL). The pH of the aqueous layer was 1. The organic layer was washed with water, saturated sodium bicarbonate and brine, dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was dissolved in hot dichloromethane (50 mL). Ethyl acetate was added (50 mL). The product precipitated. The solution was boiled for 5 minutes, cooled to room temperature, and hexanes were added (50 mL). The resulting crystals were filtered, then washed with ethyl acetate (20 mL) followed by hexanes (50 mL) to yield the title compound (7.22 g, 27%) as off-white crystals. R_f = 0.27 (silica gel, 10% isopropanol in dichloromethane). The title compound also was prepared by the route described in Example 102.

Example 31

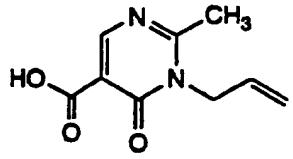
Preparation of ethyl 1-allyl-2-methyl-pyrimidin-6(1H)-one-5-carboxylate



Using similar procedures to that described above in Example 21, the title compound is prepared from the compound of Example 30.

Example 32

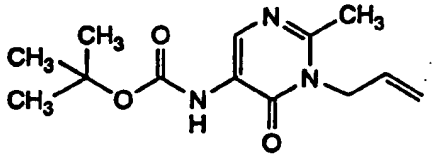
Preparation of 1-allyl-2-methyl-pyrimidin-6(1H)-one-5-carboxylic acid



Using similar procedures to that described above in Example 22, the title compound is prepared from the compound of Example 31.

Example 33

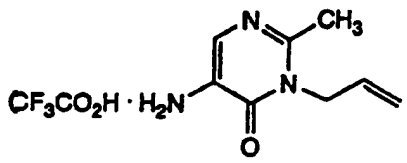
Preparation of 1-allyl-2-methyl-5-t-butyloxycarbonylamino-6-oxo-1-pyrimidi-6(1H)-one



Using similar procedures to that described above in Example 23 the title compound is prepared from the compound of Example 32.

Example 34

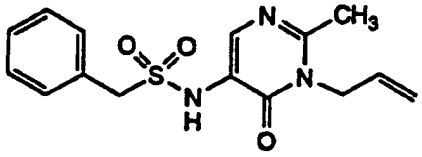
Preparation of 1-allyl-2-methyl-5-amino-6-oxo-1-pyrimidi-6(1H)-one, trifluoroacetate salt



Using similar procedures to that described above in Example 24, the title compound is prepared from the compound of Example 33.

Example 35

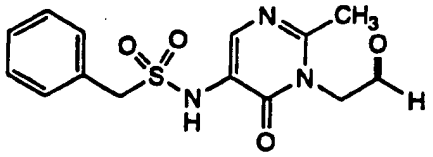
Preparation of 1-allyl-2-methyl-5-benzylsulfonylamino-6-oxo-1-pyrimidi-6(1H)-one



Using similar procedures to that described above in Example 25, the title compound is prepared from the compound of Example 34.

Example 36

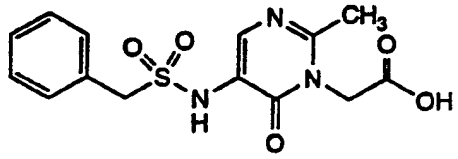
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetaldehyde



Using similar procedures to that described above in Example 26, the title compound is prepared from the compound of Example 35.

Example 37

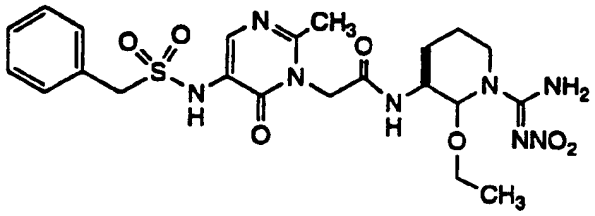
Preparation of 5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetic acid



Using similar procedures to that described above in Example 27, the title compound is prepared from the compound of Example 36. An alternative method of preparing the title compound is described in Examples 102 to 107.

Example 38

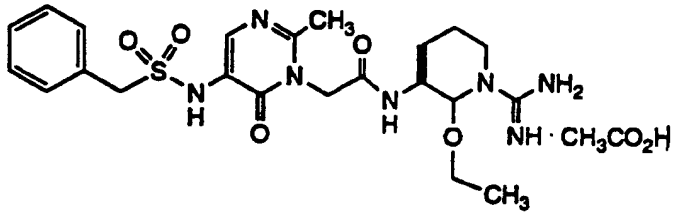
Preparation of 2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-N^{g}-nitro-L-argininal ethyl cyclol



Using similar procedures to that described above in Example 8, the title compound is prepared from the compound of Example 37.

Example 39

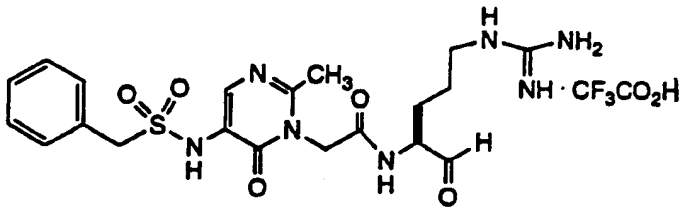
Preparation of 2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal ethyl cyclol, acetate salt



Using similar procedures to that described above in Example 9, the title compound is prepared from the compound of Example 38.

Example 40

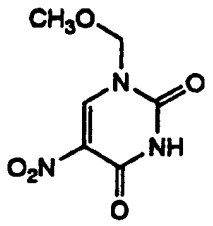
Preparation of 2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal, trifluoroacetate salt



Using similar procedures to that described above in Example 10, the title compound is prepared from the compound of Example 39. An alternative method of preparing the title compound is described in Example 113 (Compound D).

Example 41

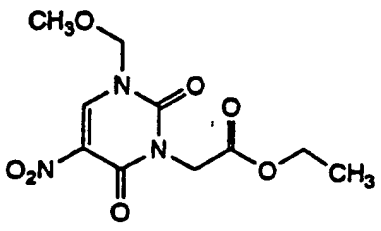
Preparation of 5-nitro-1-methoxymethyluracil



5-nitrouracil (10.00 g, 64 mmole), 1,1,1-3,3,3-hexamethyldisilazane (40 mL, 190 mmole) and chlorotrimethylsilane (4.0 mL, 32 mmole) are heated to reflux for 24 hours. The solution is concentrated under reduced pressure to afford 5-nitrouracil bis(trimethylsilyl) ether. 5-nitrouracil bis(trimethylsilyl) ether (10.0 g, 24 mmole), dimethylformamide (50 mL) and bromomethylmethyl ether (5.9 mL, 73 mmole) are heated in an 80°C oil bath for 24 hours. Ice water (500 mL) is added, and the mixture is stirred for 30 minutes, then extracted with dichloromethane (3X). The combined organic layers are dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give the title compound.

Example 42

Preparation of ethyl 5-nitro-1-methoxymethyl-3-uracilylacetate

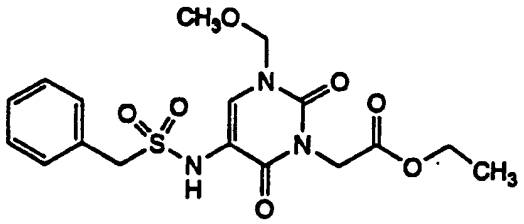


The compound of Example 41 (10.0 g, 50 mmole) is dissolved in tetrabutylammonium flouride (120 mL of a 1.0M solution in tetrahydrofuran, 0.124 mole). Ethyl bromoacetate (8.3 mL, 75 mmole) is added. The reaction

mixture is stirred at ro m temp rature. The reaction mixture is concentrated, then partitioned between dichloromethane and water. The aqueous layer is extracted with dichloromethane. The combined organic extracts are washed with water, brine, and dried over anhydrous magnesium sulfate. The solvent is removed under vacuum to afford the title compound.

Example 43

Preparation of ethyl 5-benzylsulfonylamino-1-methoxymethyl-3-uracilylacetate

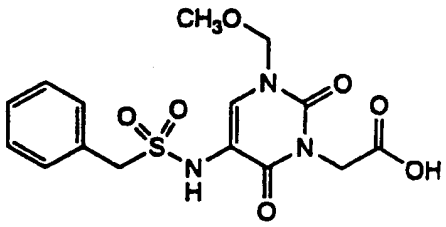


A stirred solution of the compound of Example 42 (10.0 g, 35 mmole) in ethanol (100 mL) is hydrogenated over 10% Pd/C (1.00 g) for 8 hours under balloon pressure. Celite is added, and the reaction mixture is filtered through a pad of celite, using ethyl acetate to wash. The solvent is removed *in vacuo* to give crude ethyl 5-amino-1-methoxymethyl-uracilylacetate.

A stirred solution of ethyl 5-amino-1-methoxymethyl-uracilylacetate (8.0 g, 31 mmole) and 2,4,6-collidine (13.7 mL, 62 mmole) in tetrahydrofuran (50 mL) is cooled in an ice bath. A solution of benzylsulfonyl chloride (5.93 g, 31 mmole) in tetrahydrofuran (50 mL) is added over a 30-minute period. After addition is complete, the solution is stirred for 1 hour at 0°C, then 3 hours at room temperature. The reaction mixture is diluted with ethyl acetate, washed with 1.0N HCl (until aqueous layer is pH 1), water, saturated sodium bicarbonate, and brine. The rganic layer is dried over anhydrous magnesium sulfate, and the solvent is removed. The title compound is isolated.

Example 44

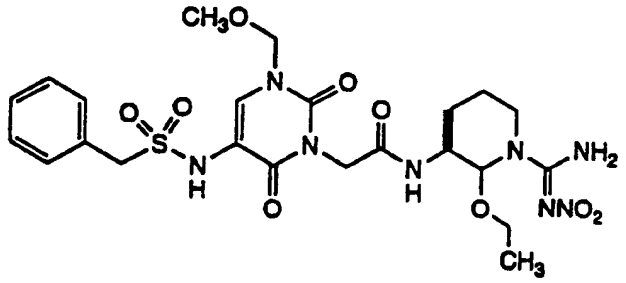
Preparation of 5-benzylsulfonylamino-1-methoxymethyl-3-uracilylacetic acid



To a cooled (0°C) suspension of the compound of Example 43 (10.0 g, 24 mmole) in methanol (50 mL) is added 1.0N NaOH (49 mL) over a period of 10 minutes. After the addition is complete, the solution is allowed to warm to room temperature over a period of 1.5 hours. The solvent is reduced under vacuum, residue diluted with water, and washed with ethyl acetate twice. The aqueous layer is acidified with 2.0N HCl to pH 1, extracted with ethyl acetate three times. The combined organic extracts are washed with water, then brine (twice). The solvent is removed to give the title compound.

Example 45

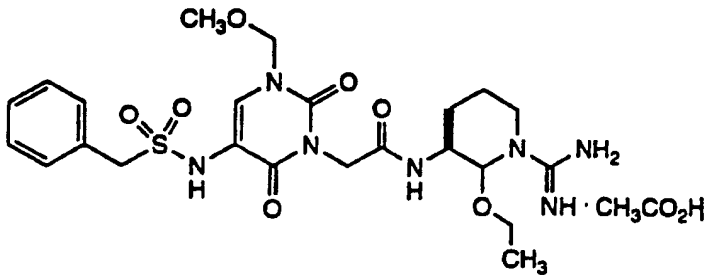
Preparation of 5-benzylsulfonylamino-1-methoxymethyl-3-uracilylacetyl-N^{g}-nitro-L-argininal ethyl cyclol



To a stirred suspension of the compound of Example 44 (10.0 g, 26 mmole), N^{g}-nitro-L-argininal ethyl cyclol, hydrochloride salt (8.38 g, 31 mmole), and N-hydroxybenzotriazole (4.0 g, 26 mmole) in acetonitrile (200 mL) cooled to 0°C is added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (6.0 g, 31 mmole). N-methylmorpholine (8.6mL, 78 mmole) is added dropwise. After the addition is complete, the reaction is stirred at room temperature for 3 hours. The solvent is reduced under vacuum, and the resulting residue is dissolved in dichloromethane, washed with 2.0N HCl (to pH 1), water, saturated sodium bicarbonate and brine. The extract is dried over anhydrous magnesium sulfate, and the solvent is removed under vacuum. The crude product is chromatographed through silica gel to yield the title compound.

Example 46

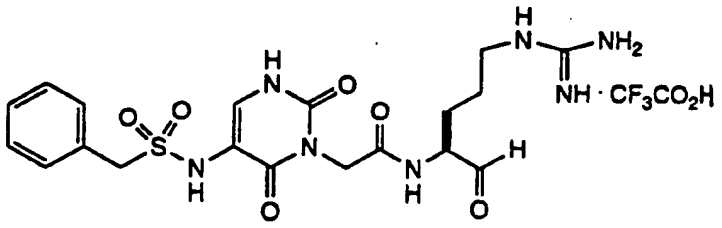
Preparation of 5-benzylsulfonylamino-1-methoxymethyl-3-uracilylacetyl-L-argininal ethyl cyclol, acetate salt



The compound of Example 45 (1.00 g) in ethanol/acetic acid/water (4:1:1, 10 mL) is hydrogenated over 10% palladium on carbon (0.30 g) for 4 hours at 20 psi. Celite is added, and the solution is filtered through a 0.2 micron filter, washing the solid with ethanol/acetic acid/water (4:1:1, 10 mL). To the filtrate is added 10% palladium on carbon (0.30 g), and the solution is hydrogenated at 20 to 25 psi until there is no starting material as observed by analytical HPLC. Celite is added, and the solution was filtered through a 0.2 micron filter, washing the solid with water. The solvent is reduced to a volume of 80 mL under reduced pressure, then washed with ethyl acetate. The solvent from the aqueous layer is reduced to remove the volatiles, then the sample is lyophilized to yield the title compound.

Example 47

Preparation of 5-benzylsulfonylamino-3-uracilylacetyl-L-argininal, trifluoroacetate salt

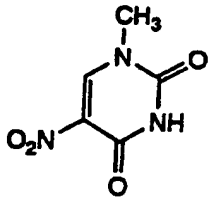


The compound of Example 46 (1.00 g) is dissolved in

3.0N hydrochloric acid (20 mL). After 3 hours, the reaction mixture is quenched with aqueous sodium acetate (to pH 3.5), then filtered through a 2 micron filter. The filtrate is purified by preparative HPLC (5x25 cm Vydac C-18 column, 0 to 20% acetonitrile/water containing 0.1% trifluoroacetic acid). The clean fractions, as analyzed by analytical HPLC, are combined to give the title compound.

Example 48

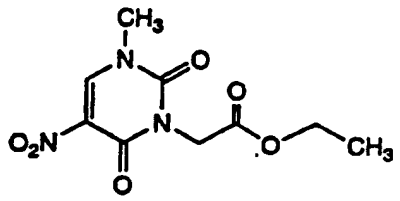
Preparation of 5-nitro-1-methyl-uracil



5-nitrouracil (10.00 g, 64 mmole), 1,1,1-3,3,3-hexamethyldisilazane (40 mL, 190 mmole) and chlorotrimethylsilane (4.0 mL, 32 mmole) are heated to reflux for 24 hours. The solution is concentrated under reduced pressure to afford 5-nitrouracil bis - (trimethylsilyl) ether. 5-nitrouracil bis(trimethylsilyl) ether (10.0 g, 24 mmole), dimethylformamide (50 mL) and iodomethane (3.0 mL, 49 mmole) are heated in an 80°C oil bath for 24 hours. Ice water is added, and the mixture is stirred for 30 minutes, then extracted with dichloromethane (3X). The combined organic layers are dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give the title compound. An alternative method of preparing the title compound is described in Example 108.

Example 49

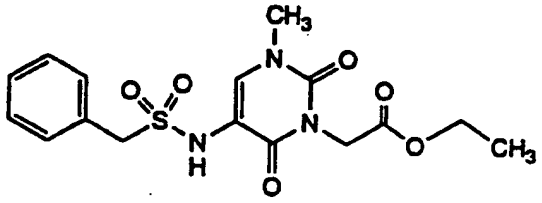
Preparation of ethyl 5-nitro-1-methyl-3-uracilylacetate



Using similar procedures to that described above in Example 42, the title compound is prepared from the compound of Example 48.

Example 50

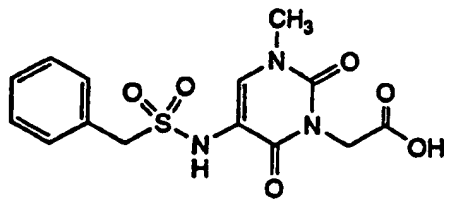
Preparation of Ethyl 5-benzylsulfonylamino-1-methyl-3-uracilylacetate



Using similar procedures to that described above in Example 43, the title compound is prepared from the compound of Example 49.

Example 51

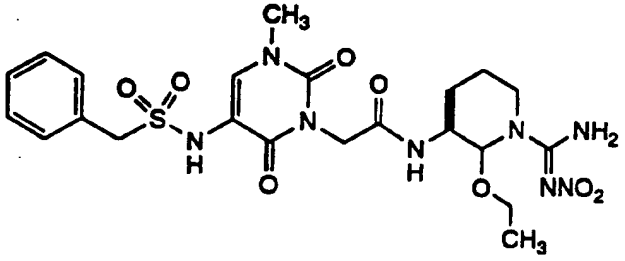
Preparation of 5-benzylsulfonylamino-1-methyl-3-uracilylacetic acid



Using similar procedures to that described above in Example 44, the title compound is prepared from the compound of Example 50. An alternate method of preparing the title compound is described in Example 111.

Example 52

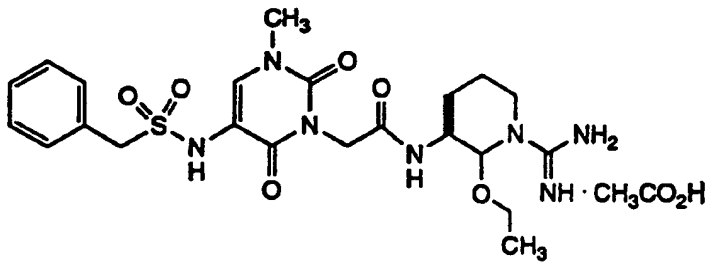
Preparation of 5-benzylsulfonylamino-1-methyl-3-uracilylacetyl-N^{g}-nitro-L-argininal ethyl cyclol



Using similar procedures to that described above in Example 45, the title compound is prepared from the compound of Example 51.

Example 53

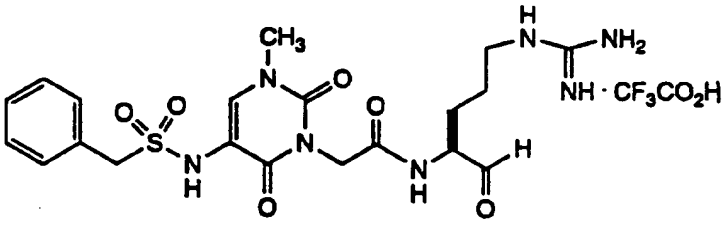
Preparation of 5-benzylsulfonylamino-1-methyl-3-uracilylacetyl-L-argininal ethyl cyclol, acetate salt



Using similar procedures to that described above in Example 46, the title compound is prepared from the compound of Example 52.

Example 54

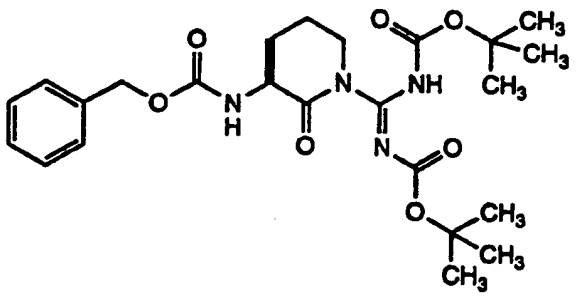
Preparation of 5-benzylsulfonylamino-1-methyl-3-uracilylacetyl-L-argininal, trifluoroacetate salt



Using similar procedures to that described above in Example 47, the title compound is prepared from the compound of Example 53. An alternative method of preparing the title compound is described in Example 113 (Compound E).

Example 55

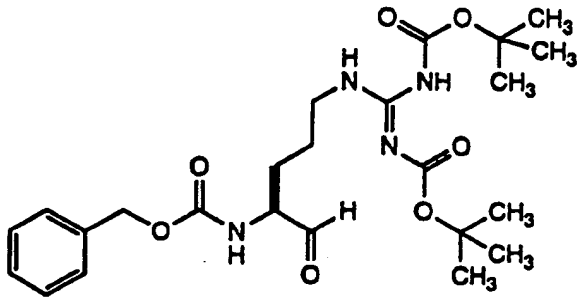
Preparation of alpha-N-benzyloxycarbonyl-omega,omega'-di-N-t-butoxycarbonyl-L-arginine lactam



Alpha-N-t-benzyloxycarbonyl-omega,omega'-di-N-t-butoxycarbonylarginine (2.10 g. 4.1 mmole)) was dissolved in acetonitrile (25 mL). Hydroxybenzotriazole (0.63 g, 4.1 mmole) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (0.79 g, 4.1 mmole) were added in succession. After the reaction was stirred for 1 hour, the solvent was reduced. The residue was dissolved in ethyl acetate (50 mL), washed with water, saturated sodium bicarbonate, and brine. The solution was dried over anhydrous magnesium sulfate, filtered, and the solvent was removed *in vacuo* to give 1.90 g (94% yield) of the title compound. R_f = 0.37 (10% ethyl acetate/dichloromethane).

Example 56

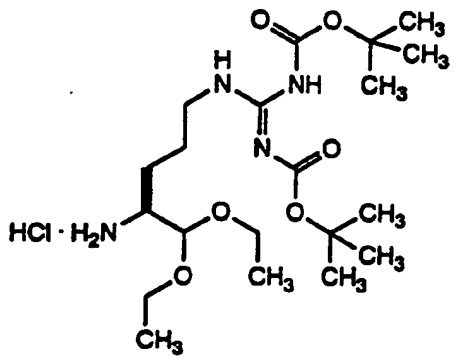
Preparation of alpha-N-benzyloxycarbonyl-omega,omega'-di-N-t-butoxycarbonyl-L-argininal



A solution of the compound of Example 55 (33.69 g, 69 mmole) in tetrahydrofuran (350 mL) was cooled to -70°C. Lithium aluminum hydride (69 mL of a 1.0M solution in tetrahydrofuran) was added dropwise while maintaining the temperature below -60°C. After the reaction mixture was stirred at -60 to -65°C for 30 minutes, it was cooled to -70°C. 2.5 M potassium bisulfate (92 mL) was added dropwise to quench the excess lithium aluminum hydride. The solution was allowed to warm to 0°C, and the mixture was filtered through celite, washing with ethyl acetate. The filtrate was washed with cold 1.0N HCl (75 mL), ice water (50 mL), cold saturated sodium bicarbonate (50 mL), then cold brine (50 mL). The extract was diluted with dichloromethane (200 mL), then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* to afford 28.23 g.(83%) of the title compound. R_f = 0.37 (5% isopropanol in dichloromethane).

Example 57

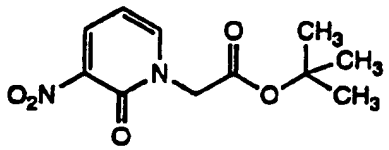
Preparation of omega,omega'-di-N-t-butoxycarbonyl-L-argininal diethyl acetal, HCl salt



The compound of Example 56 (300 mg, 0.61 mmole) was dissolved in ethanol (3.0 mL) and concentrated HCl was added (51 microliters). After stirring overnight at room temperature, 10% Pd/C (30 mg) was added. The mixture was hydrogenated for 4 hours. TLC indicated that the reaction was complete. Celite was added, and the reaction mixture was filtered. The solution was diluted with water to a volume of 50 mL. The title compound (190 mg, 73% yield) was freeze dried to a yellow solid. R_f = 0.26 (10% methanol/dichloromethane).

Example 58

Preparation of t-butyl (3-nitro-2-oxo-1,2-dihydropyridyl)acetate

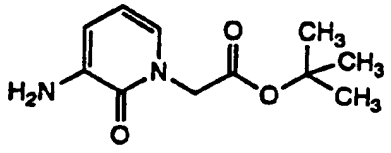


Sodium hydride (1.57 g of a 60% dispersion in mineral oil, 0.039 mole) was washed with hexanes three times (10 mL each) and suspended in dimethylformamide (25 mL). The stirred suspension was cooled in an ice bath,

then 3-nitro-2-hydroxypyridine (5.00 g, 0.036 mole) was added in small portions over a 25-minute period. After the addition was complete, the reaction was stirred at 0°C for 10 minutes, then room temperature for 30 minutes. The reaction mixture was recooled in an ice bath. t-Butyl bromoacetate (5.25 mL, 0.036 mole) was added. The reaction was stirred at 0°C for 1 hour, then 1.5 hours at room temperature. The reaction mixture was diluted with ethyl acetate (80 mL), and ice (80 g) was added. The aqueous layer was extracted with ethyl acetate (3x200 mL). The combined organic extracts were washed with water (4x100 mL), brine (100 mL), and dried over anhydrous sodium sulfate. The solvent was removed, and the resulting residue was chromatographed through silica gel using 10% ethyl acetate/dichloromethane as eluent. The pure fractions were combined, and the solvent was removed under vacuum to afford 6.77 g (75% yield) of the title compound as yellow solid. R_f = 0.30 (silica gel, 20% ethyl acetate in dichloromethane).

Example 59

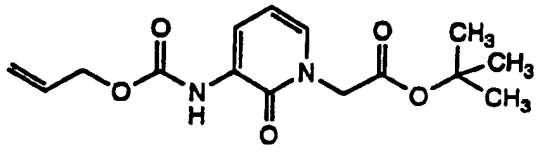
Preparation of t-butyl (3-amino-2-oxo-1,2-dihydropyridyl)acetate



A stirred solution of the compound of Example 58 (2.00 g, 7.9 mmole) in ethanol (50 mL) was hydrogenated over 10% Pd/C (0.23 g) for 3 hours under balloon pressure. Celite was added, and the reaction mixture was filtered through a pad of Celite, using methanol/ethyl acetate to wash. The solvent was removed under vacuum to afford the title compound (1.90 g) in quantitative yield. R_f = 0.56 (silica gel, 10% methanol in dichloromethane).

Example 60

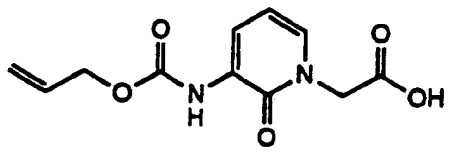
Preparation of ethyl 3-[(allyloxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetate



The compound of example 59 (1.7 g, 7.85 mmole) is dissolved in 50% aqueous dioxane (30 mL) and cooled to 0°C. Sodium bicarbonate (2.0 g, 24 mmole) is added in one portion. After stirring 5 minutes, allyl chloroformate (1.67 g, 16 mmole) in dioxane (4 mL) is added dropwise over a 5-minute period. After stirring for 30 minutes, the solvent is reduced to a volume of 10 mL, and extracted with dichloromethane (50 mL). The organic layer is washed with brine, the dried over anhydrous magnesium sulfate. The solvent is removed and the title compound isolated.

Example 61

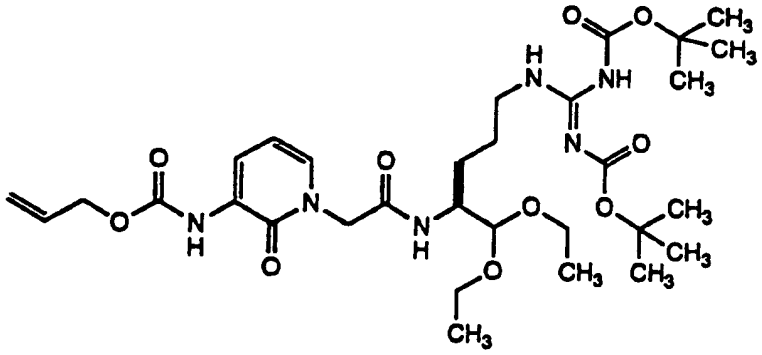
Preparation of 3-[(allyloxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetic acid



The compound of example 60 (1.00 g) is treated with 50% trifluoroacetic acid in dichloromethane (10 mL) for 1 hour at 0°C, and 3 hours at room temperature. The solution is diluted with toluene (50 mL) and the solvent is removed *in vacuo* to afford the title compound.

Example 62

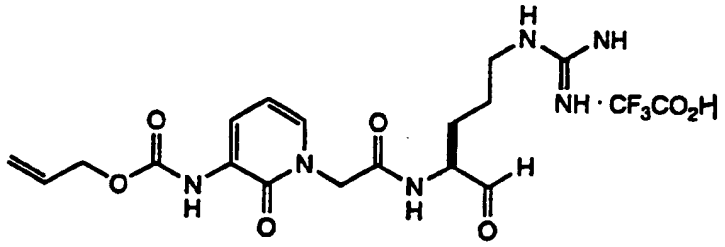
Preparation of 3-[(allyloxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-omega,omega'-di-N-t-butoxycarbonyl-L-argininal diethyl acetal



To a stirred suspension of the compound of Example 57 (500 mg, 1.98 mmole), the compound of example 61 (1.12 g, 2.37 mmole), and N-hydroxybenzotriazole (300 mg, 1.98 mmole) cooled to 0°C, is added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (457 mg, 2.38 mmole). N-methylmorpholine (0.65 mL, 5.9 mmole) is added dropwise. After the addition is complete, the reaction is stirred at room temperature overnight. The solvent is reduced under vacuum, and the resulting residue is dissolved in ethyl acetate, washed with 1.0N HCl (to pH 1), water, saturated sodium bicarbonate and brine. The extract is dried over anhydrous magnesium sulfate, and the solvent is removed under vacuum to yield the title compound.

Example 63

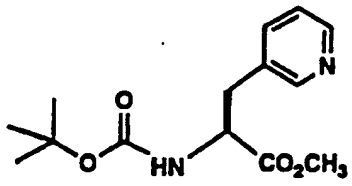
Preparation of 3-[(allyloxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal, trifluoroacetate salt



The compound of example 62 (1.0 g) is suspended in 50% aqueous acetonitrile (20 ml) and cooled in an ice bath. Hexafluorophosphoric acid (60% by weight, 10 mL) is added slowly, and the cooling bath is removed. After 30 minutes, the reaction mixture is recooled in an ice bath, and quenched with aqueous sodium acetate (2.5M solution) to pH 4, then filtered through a 2 micron filter. The filtrate is purified using preparative HPLC. The fractions are analyzed for purity by analytical HPLC (0.1% trifluoroacetic acid/10-40% aqueous acetonitrile), combined, and the acetonitrile is removed under reduced pressure. The remaining water is lyophilized. The title compound is recovered.

Example 64

Preparation of N-(t-butoxycarbonyl)-3-(3-pyridyl)-L-alanine methyl ester

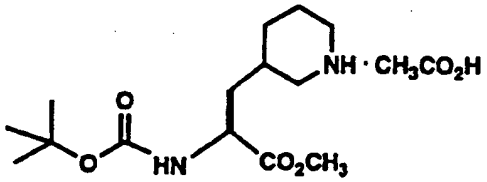


To a solution of N-(t-butoxycarbonyl)-3-(3-pyridyl)alanine (5.0 g, 18.8 mmole) in methanol (100 mL) was added thionyl chloride (2M solution in

dichloromethane, 66 mL, 132 mmole). The resulting solution was stirred overnight at ambient temperature. The methanol was removed under reduced pressure to a minimum volume and ethyl acetate (100 mL) was added. The resulting white precipitate was collected in a fritted funnel. To a solution of the collected precipitate in a mixture of tetrahydrofuran/water (40 mL each) was added di-tert-butyl dicarbonate (4.8 g, 21.99 mmole) and sodium carbonate (1.95 g, 18.4 mmole). After stirring for 12 hours at ambient temperature, the reaction mixture was diluted with ethyl acetate (40 mL) and washed with a solution of saturated sodium bicarbonate (25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum to give crude product. This product was subjected to flash column chromatography on silica gel (230-400 mesh) using a 8x52 cm column and eluting with a 10:90 mixture of ethyl acetate/hexane followed by a 60:40 mixture of ethyl acetate/hexane. 4 g (74%) of the title compound was obtained as an oil. Thin-layer chromatography gave a R_f = 0.68 (silica gel; ethyl acetate).

Example 65

Preparation of N-(t-butoxycarbonyl)-3-(3-piperidyl)-L-alanine methyl ester, acetate salt

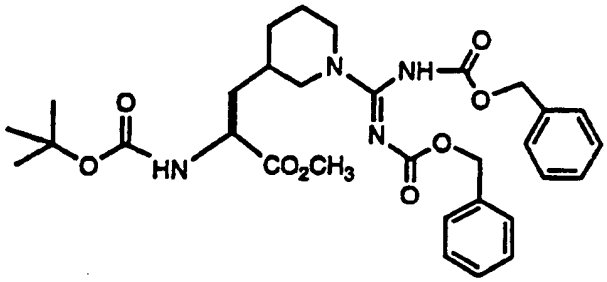


A solution of the compound of Example 64 (5 g, 17.8 mmole) in ethanol (24 mL), acetic acid (6 mL) and water (6 mL) was hydrogenated over platinum oxide (500 mg) at 45 psi for three hours. The catalyst was filtered off and the filtrate concentrated under vacuum to an oily residue (6.89 g) which was used in the next step (Example 6) without further purification. Thin-layer chromatography

yielded two spots corresponding to two diastereomers with R_f values of 0.16 and 0.26, respectively (silica gel; 4:1:1 n-butanol/ acetic acid/water).

Example 66

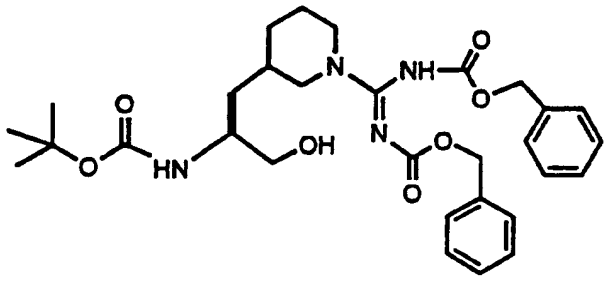
Preparation of N-(t-butoxycarbonyl)-3-[3-piperidyl-(N-guanidino (bis-benzyloxycarbonyl))]-L-alanine methyl ester



To a solution of the compound of Example 65 (6.89 g, 19.9 mmole) in tetrahydrofuran (80 mL) was added S-methylisothiourea bis-benzyloxycarbonyl (7.13 g, 19.9 mmole) followed by N-methylmorpholine (4.37 mL), and the reaction mixture was stirred at ambient temperature for 18 hours. The reaction mixture then was concentrated under vacuum and the resulting residue was dissolved in ethyl acetate (100 mL) and washed with 1N sodium bisulfate and saturated sodium chloride (50 mL each). After drying over anhydrous sodium sulfate, the solvents were removed under vacuum; the crude title compound was subjected to flash column chromatography on silica gel (230-400 mesh) using a 8x52 cm column and eluting with 1:9 ethyl acetate/hexanes (two column volumes) followed by 1:1 ethyl acetate/hexanes. 2.75 g the title compound was obtained as a mixture of two diastereomers. Thin-layer chromatography gave two spots with R_f values of 0.57 and 0.62, respectively (silica gel; 1:1 ethyl acetate/ hexanes).

Example 67

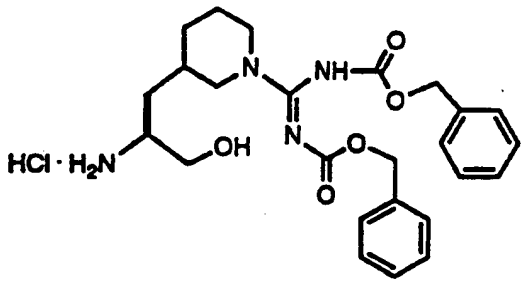
Preparation of N-(t-butoxycarbonyl)-3-[3-piperidyl-(N-guanidino (bis-benzyloxycarbonyl))]-L-alaninol



To a stirred solution of the compound of Example 66 (2.23 g, 3.7 mmole) in absolute ethanol (8 mL) and anhydrous tetrahydrofuran (4 mL) was added calcium chloride (844 mg, 7.6 mmole) and sodium borohydride (575 mg, 15.2 mmole). After stirring 12 hours at ambient temperature, the reaction mixture was concentrated under vacuum and the resulting residue was partitioned between ethyl acetate and 1N sodium bisulfate (10 mL each). The two layers were separated; organic layer was washed twice more with 1N sodium bisulfate, dried over anhydrous sodium sulfate and concentrated under vacuum gave a residue. Flash column chromatography of the residue on silica gel (230-400 mesh) using a 5.5x45 cm column and eluting with ethyl acetate gave 1.3 g of the title compound as a white foam. Thin layer chromatography yielded two spots corresponding to two diastereomers with R_f values of 0.18 and 0.27, respectively (silica gel; 1:1 ethyl acetate/ hexanes).

Example 68

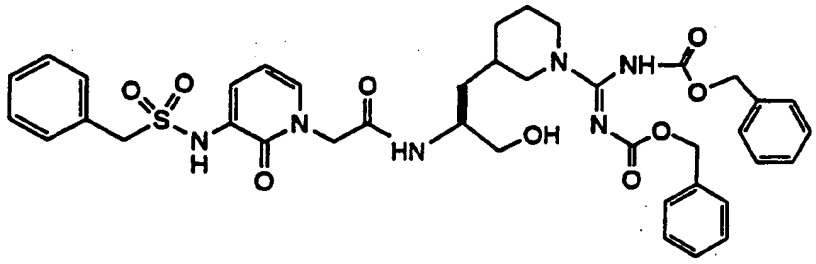
Preparation of 3-[3-piperidyl-(N-guanidino(bis-benzyloxycarbonyl)))-L-alaninol, hydrochloride salt



The compound of Example 67 (290 mg, 0.57 mmole) was treated with 2.5 N anhydrous hydrochloric acid in ethyl acetate (2.0 mL) at ambient temperature for one hour. The solvent was removed under vacuum to give a sticky-white solid (260 mg). This solid was used in the next step (Example 20) without further purification. ^1H NMR spectrum taken in CD_3OD showed no t-butoxycarbonyl protons at 1.4 ppm.

Example 69

Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-3-[3-piperidyl-(N-guanidino(bis-benzyloxycarbonyl)))-alaninol

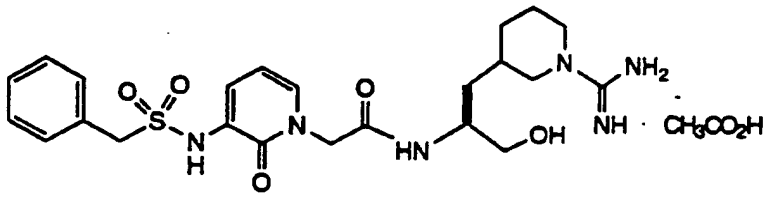


To a suspension of the compound of Example 68 (266 mg, 0.45 mmole) in acetonitrile (7 mL) is added successively the compound of Example 7 [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetic acid (145 mg, 0.41 mmole), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (86 mg, 0.45

mmole), 1-hydr xybenzotriazole hydrate (72 mg, 0.47 mmole) and diisopropylethylamine (2.44 mmole, 417 microliters). The solution is stirred at ambient temperature for twelve hours. The solvent is removed under reduced pressure and the remaining residue is dissolved in ethyl acetate (15 mL) and washed two times each with 10 mL portions of 1N sodium bisulfate, saturated sodium bicarbonate and saturated sodium chloride solution. The organic layer is dried over sodium sulfate and concentrated to crude product. The title compound is isolated.

Example 70

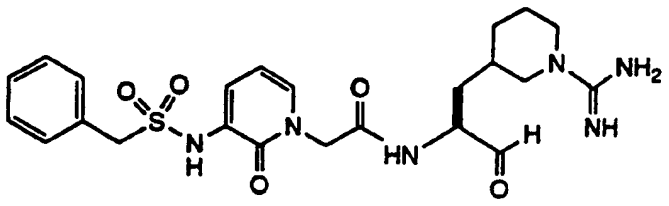
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-3-[3-piperidyl-(N-guanidino)] alaninol, acetate salt



The compound of Example 69 (123 mg, 0.16 mmole) is subjected to catalytic hydrogenation in methanol (8 mL), and acetic acid (2 mL) and water (2 mL) in the presence of palladium on carbon (20 mg) at 40 psi for 4 hours. The title compound is obtained.

Example 71

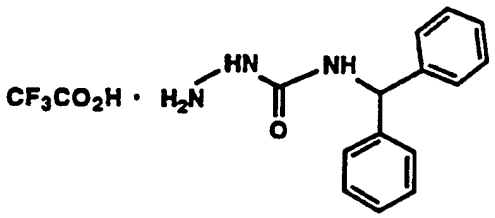
Preparation of [3-[[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-3-[3-piperidyl-(N-guanidino)]alaninal



To a chilled solution of the compound of Example 70 (107 mg, 0.19 mmole) in dimethylsulfoxide and toluene (2 mL each) is added dichloroacetic acid (78 microliter, 0.94 mmole) followed by 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (0.36 g, 1.9 mmole) at one minute later. The reaction is stirred for 5 minutes at 0°C and 85 minutes at ambient temperature, and quenched with 30 mL water. The water layer is extracted twice with diethyl ether (15 mL portions), diluted to 60 mL with water and is purified by high pressure liquid chromatography using a reverse phase column containing a C-18 resin comprised of 10 micron-size gel particles with a 300 angstrom pore size. The column is eluted with a water/acetonitrile (containing 0.1% trifluoroacetic acid) gradient where the gradient is run from 10% to 30% acetonitrile. Each diasteromer of the title compound is isolated.

Example 72

Preparation semicarbazid-4-yl diphenylmethane, trifluoroacetate salt



Step 1

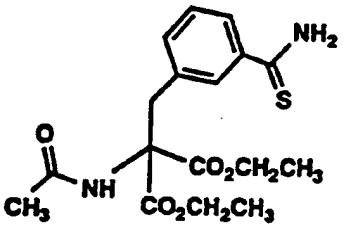
A solution of carbonyldiimidazole (16.2 g, 0.10 mole) in 225 mL of dimethylformamide was prepared at room temperature and allowed to stir under nitrogen. A solution of t-butyl carbazate (13.2 g, 0.100 moles) in 225 mL dimethylformamide was then added dropwise over a 30 minute period. Next, diphenylmethylamine (18.3 g, 0.10 moles) was added over a 30 minute period. The reaction was allowed to stir at room temperature under nitrogen for one hour. Water (10 mL) was added and this mixture was concentrated to about 150 mL under vacuum. This solution was poured into 500 mL water and extracted with 400 mL of ethyl acetate. The ethyl acetate phase was extracted two times each with 75 mL 1N HCl, water, saturated sodium bicarbonate and brine, and then was dried with anhydrous magnesium sulfate. The mixture was filtered and the solution was concentrated to give 29.5 g (85% yield) of 1-t-butoxycarbonyl-semicarbazid-4-yl diphenylmethane as a white foam. This material may be purified by recrystallization from ethyl acetate/hexane, but was pure enough to use directly in step 2: mp 142-143°C. ^{1}H NMR ($CDCl_3$) delta 1.45 (s, 9H), 6.10 (dd, 2H), 6.42 (s, 1H), 6.67 (bs, 1H), 7.21-7.31 (m, 10H). Analysis Calculated for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.31. Found: C, 66.46; H, 6.75; N; 12.90.

Step 2

A solution of 3.43 g (10 mmole) of 1-t-butoxycarbonyl-semicarbazid-4-yl diphenylmethane in 12.5 mL of dichloromethane was treated with 12.5 mL of trifluoroacetic acid at 0°C. The reaction mixture was allowed to stir for 30 minutes at this temperature. The reaction mixture was then added dropwise to 75 mL of diethyl ether to give a precipitate. The resulting precipitate was filtered off and washed with diethyl ether to give 2.7 g (80% yield) of the title compound; mp 182-184°C.

Example 73

Preparation of 3-thioamidobenzyl-N-acetylaminomalonic acid diethyl ester



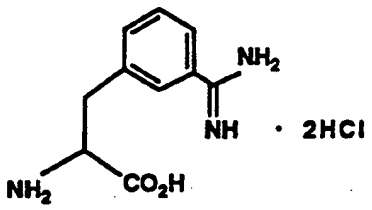
To a stirred solution of alpha-bromo-meta-tolunitrile (45.0 g, 0.24 mole), diethyl acetamidomalonate (48.0 g, 0.22 mole) and potassium iodide ((3.0 g, 0.018 mole) in dioxane (500 mL) was added 2.5M sodium ethoxide in ethanol (100 mL) dropwise under an argon atmosphere. After the addition was complete, the solution was refluxed for 6 hours. The reaction mixture was allowed to stand overnight at room temperature, then diluted with brine (250 mL) and water (250 mL), and extracted with ethyl acetate four times (1.0 L total). The combined extracts were washed with water (100 mL), 10% citric acid (100 mL), water (100 mL) and brine (2x50 mL), then dried over anhydrous magnesium sulfate and filtered; the solvent was removed under vacuum. The crude residue was recrystallized from ethyl acetate and diethyl ether in two crops to yield 43.51 g (60%) of the 3-cyanobenzyl-N-

acetylaminomalonic acid diethyl ester as yellow crystals.

$H_2S(g)$ was bubbled into a rapidly stirring solution of 3-cyanobenzyl-N-acetylaminomalonic acid diethyl ester (44.3 g, 0.13 mmole) in pyridine (300 mL) and triethylamine (100 mL) for 40 minutes. The reaction mixture was stirred at room temperature for 16 hours, then poured into 3.0 L of water. A yellow precipitate formed immediately. The solution was allowed to stand at 4°C for 4 hours, then was filtered. The crude title compound was recrystallized from ethyl acetate and hexanes to yield 48.1g (98%) of the title compound as yellow crystals. m.p. 183-186°C. ^1H NMR ($CDCl_3$): delta 1.31 (t, J=7.1 Hz, 6H), 2.06 (s, 3H), 3.70 (s, 2H), 4.29 (q, J=7.1 Hz, 4H), 4.80-4.87 (m, 1H), 6.60 (s, 1H), 7.10-7.20 (m, 1H), 7.27-7.35 (m, 2H), 7.60-7.70 (m, 2H). Analysis Calculated for $C_{17}H_{22}N_2O_5S$: C, 55.72; H, 6.05; N, 7.64. Found: C, 55.55; H, 5.96; N, 7.76.

Example 74

Preparation of 3-amidino-D,L-phenylalanine, dihydrochloride salt

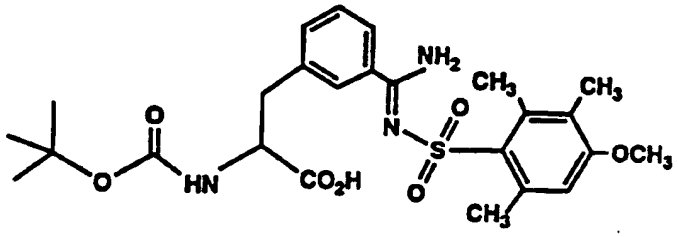


The compound of Example 73 (48.1 g, 0.13 mmole) was dissolved in acetone (800 mL). Iodomethane (18.3 mL, 0.19 mole, 1.5 equivalents) was added, and the solution was refluxed for 30 minutes. The solution was cooled to room temperature, and the intermediate thioimidate was filtered, dried and dissolved in methanol (500 mL). Ammonium acetate (14.8 g, 0.19 mole, 2 equivalents) was added. The reaction mixture was refluxed for 1 hour, then cooled to room temperature, and poured into ether (1.2 L). The solution was allowed to stand at 4°C for 72 hours.

The crude 3-amidinobenzyl-N-acetylaminomalonic acid diethyl ester was filtered, washed with ether, air dried, and then refluxed in concentrated HCl (250 mL) for 3 hours. The reaction mixture was concentrated under vacuum, diluted with water (0.5 L), and concentrated under vacuum again. These steps were repeated. The crude title compound was purified by cation-exchange (Sephadex SP-C25) using a gradient of 0-1.0N HCl as eluent to yield 10.8g (30%) of the title compound as an off-white solid. ^1H NMR (D_2O): delta 3.14-3.29 (2H, m), 4.17 (dd, J=7.4, 6.2 Hz, 1H), 7.42-7.69 (4H, m). Analysis Calculated for $C_{10}H_{13}N_3O_2 \cdot 2HCl \cdot 1.9H_2O$: C, 38.20; H, 6.03; N, 13.36. Found: C, 38.51; H, 5.64; N, 12.89.

Example 75

Preparation of N-alpha-Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-3-amidino-D,L-phenylalanine

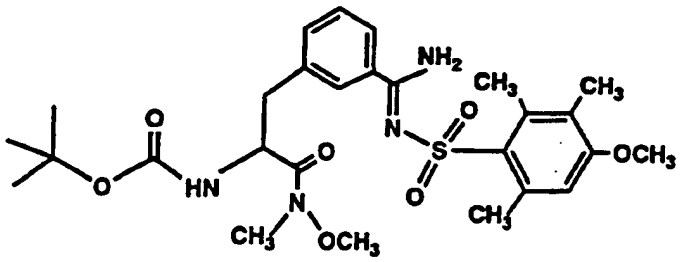


The compound of Example 74 (3-amidino-D,L-phenylalanine) (4.00 g, 13 mmole) was dissolved in 50% aqueous dioxane (20 mL). Sodium bicarbonate (3.38 g, 40 mmole) was added, followed by di-t-butyl dicarbonate (2.93 g, 13 mmole) in dioxane (4 mL). The reaction mixture was stirred for 18 hours at room temperature. The solution was cooled in an ice bath, and 4.0 N sodium hydroxide was added dropwise until the solution was pH 12. 4-methoxy-2,3,6-trimethylbenzenesulfonyl chloride (8.01 g, 32 mmole) in dioxane (10 mL) was added dropwise. 4.0 N sodium hydroxide was added as needed to keep the pH at 12. The ice bath was removed. After 1 hour, 1.0 N HCl was added to bring the solution to pH 7 to 8. The solution was diluted with an additional 50 mL of water and then was

washed with ethyl acetate two times (20 mL each). The aqueous layer was acidified to pH 1.0 with 1.0 N HCl and extracted with ethyl acetate three times (100 mL total). The combined organic layers were washed with water (20 mL) and brine twice (10 mL each). The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The residue was dissolved in a minimum amount of dichloromethane, then added dropwise to ether (25 mL). Solid impurities were removed by filtering and the solvent removed from the filtrate under vacuum to give 4.90 g (68% crude yield) of the title compound as an off-white foam. A 30 mg sample of the title compound was further purified by preparative thin-layer chromatograph developing with 1% acetic acid/5% isopropanol/ dichloromethane to give 9 mg of the title compound in a purer form. Rf = 0.16 (1% acetic acid/5% isopropanol/dichloromethane). ^{1}H NMR (CD_3OD): delta 1.32 (s, 9H), 2.14 (s, 3H), 2.63 (s, 3H), 2.71 (s, 3H), 2.93 (dd, J=13.7, 9.3 Hz, 1H), 3.22 (dd, J=13.7, 4.3 Hz, 1H), 3.85 (s, 3H), 4.34-4.37 (m, 1H), 6.72 (s, 1H), 7.35-7.47 (2H, m), 7.69-7.75 (m, 2H).

Example 76

Preparation of N-alpha-Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-3-amidino-D,L-phenylalanine-N-methyl-O-methyl-carboxamide

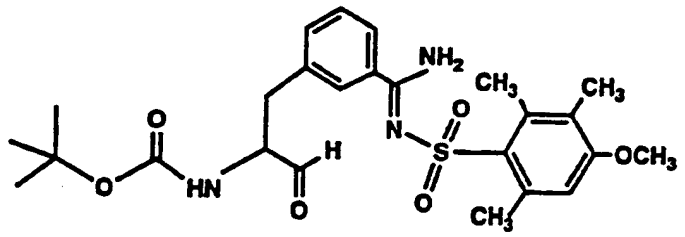


To a stirred solution of compound of Example 75 (1.00 g, 1.92 mmole), O,N-dimethyl hydroxylamine hydrochloride (375 mg, 3.85 mmole), hydroxybenzotriazole hydrate (294 mg, 1.92 mmole) and 4-methylmorpholine (1.06 mL, 9.62

mmole) in tetrahydrofuran (4 mL), co led in an ice bath, was added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (406 mg, 2.12 mmole). The ice bath was removed, and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with ethyl acetate (75 mL), washed with water, 10% citric acid, water, saturated sodium bicarbonate, and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. 750 mg (69%) of the title compound was isolated. ^1H NMR ($CDCl_3$): delta 1.33 (s, 9H), 2.14 (s, 3H), 2.66 (s, 3H), 2.75 (s, 3H), 2.80-2.88 (m, 1H), 3.06-3.20 (m, 4H), 3.70 (s, 3H), 3.84 (s, 3H), 4.98-5.06 (m, 1H), 5.21 (d, J=8.7 Hz, 1H), 6.48 (bs, 1H), 6.58 (s, 1H), 7.30-7.34 (m, 2H) 7.60-7.68 (m, 2H), 8.11 (bs, 1H). Analysis Calculated for $C_{27}H_{38}N_4O_7S \cdot 0.5H_2O$: C, 56.73; H, 6.88; N, 9.80. Found: C, 56.97; H, 6.66; N, 9.43.

Example 77

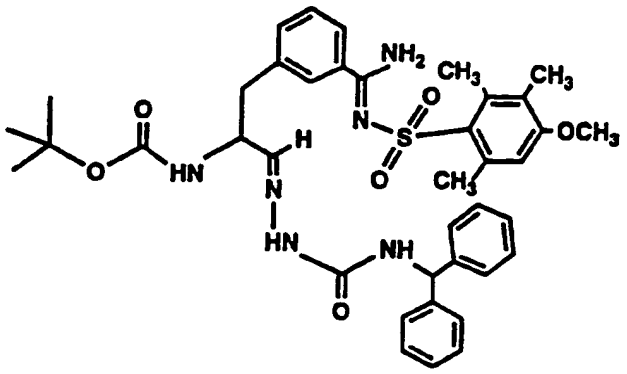
Preparation of N-alpha-Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-D,L-3-amidinophenylalaninal



To a stirred solution of $LiAlH_4$ (2.00 mL of a 1.0 M solution in tetrahydrofuran, 1.24 mmole) in tetrahydrofuran (8 mL), cooled in a dry ice/acetone bath, the compound of Example 76 (0.75 g, 1.9 mmole in tetrahydrofuran (5 mL)) was added dropwise. The cooling bath was removed and the reaction mixture was allowed to warm to 5°C. The reaction mixture was re-cooled in the dry ice acetone bath and quenched with 3.0 mL of a 1:2.7 wt./wt. solution of potassium bisulfate in water. The reaction mixture was allowed to warm to room temperature, stirred for 3 hours, filtered and concentrated under vacuum. The residue was dissolved in ethyl acetate (20 mL), and washed with 10% citric acid (2 mL), water (2 mL), saturated sodium bicarbonate (2 mL) and brine (2 mL). The organic layer was dried over anydrous magnesium sulfate and the solvent was removed under vacuum to yield 580 mg (86%) of the title compound. ^1H NMR ($CDCl_3$): delta 1.31 (s, 9H), 2.07 (s, 3H), 2.57 (s, 3H), 2.67 (s, 3H), 2.90-3.17 (2H, m), 3.77 (s, 3H), 4.33-4.40 (1H, m), 5.02-5.08 (1H, m), 6.48 (1H, s), 7.23-7.31 (2H, m), 7.50-7.62 (2H, m), 7.94, (1H, bs), 8.05 (1H, bs), 9.55 (1H, s). Analysis Calculated for $C_{25}H_{33}N_3O_6S \cdot 0.5H_2O$: C, 58.58; H, 6.69; N, 8.20. Found: C, 58.57; H, 6.72; N, 7.98.

Example 78

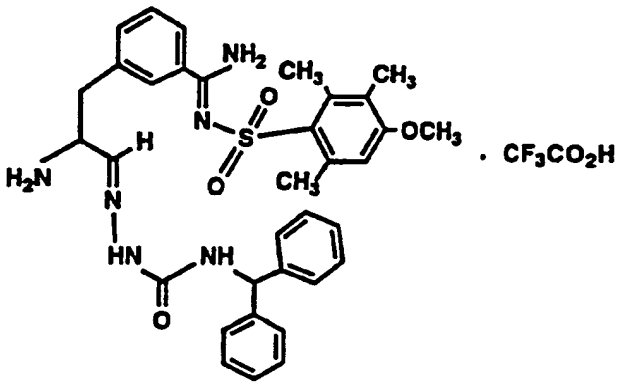
Preparation of N-alpha-Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-D,L-3-amidinophenylalaninal-semicarbazonyl-4-N-diphenylmethane



The compound of Example 77 (0.58 g, 1.9 mmole), the compound of Example 72 (410 mg, 1.15 mmole) and sodium acetate trihydrate (188 mg, 1.38 mmole) were refluxed in 75% aqueous ethanol (10 mL) for 1 hour. After the reaction mixture was cooled to room temperature, it was diluted with ethyl acetate (50 mL), washed with 1.0N HCl (5 mL), water (5 mL), saturated sodium bicarbonate (5 mL) and brine (2x5 mL), and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to yield 750 mg (89% yield) of the title compound as an off-white foam. Analysis calculated for $C_{39}H_{46}N_6O_6S \cdot 1.0H_2O$: C, 62.88; H, 6.49; N, 11.28. Found: C, 63.14; H, 6.35 N, 11.10.

Example 79

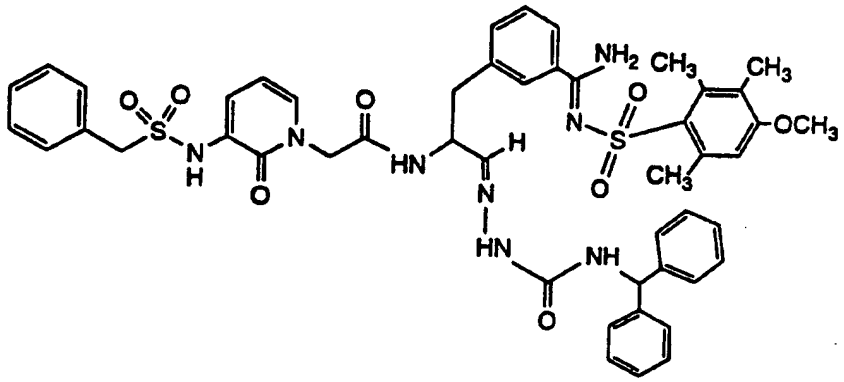
Preparation of N-omega-4-methoxy-2,3,6-trimethylbenzene sulfonyl-D,L-3-amidinophenylalaninal-semicarbazonyl-4-N-diphenylmethane, trifluoroacetate salt



The compound of Example 78 (750 mg, 1.9 mmole) was treated with 50% trifluoroacetic acid/dichloromethane (3 mL) for 30 minutes at room temperature. The reaction mixture was added dropwise to ether (50 mL). The solution was allowed to stand at 4°C for 18 hours. The product was filtered, and dried under vacuum to yield 600 mg (79% yield) of the title compound as an off-white solid. Analysis calculated for $C_{39}H_{46}N_6O_6S \cdot 1.3CF_3CO_2H$: C, 56.72; H, 5.11; N, 10.84. Found: C, 56.34; H, 5.47; N, 11.49.

Example 80

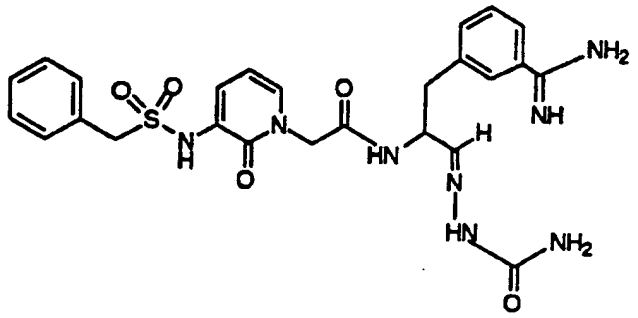
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-D,L-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-D,L-3-amidinophenyl alaninal-semicarbazonyl-4-N-diphenylmethane



1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (94 mg, 0.94 mmole) is added in one portion to a stirred solution of the compound of Example 7 (303 mg, 0.49 mmole), hydroxybenzotriazole (75 mg, 0.49 mmole), and 4-methylmorpholine (0.24 mL, 2.2 mmole) in dimethylformamide (5 mL) with cooling in an ice bath. After 30 minutes, the compound of Example 79 (363 mg, 0.49 mmole) is added. After an additional 2 hours, the reaction mixture is diluted with water (25 mL) and brine (25 mL). The product is filtered and dissolved into ethyl acetate (25 mL). The solution is washed with 10% citric acid, water, saturated sodium bicarbonate and brine, and is dried over anhydrous magnesium sulfate. The solvent is removed under vacuum. The resulting residue is chromatographed by flash chromatography on silica gel to give the title compound.

Example 81

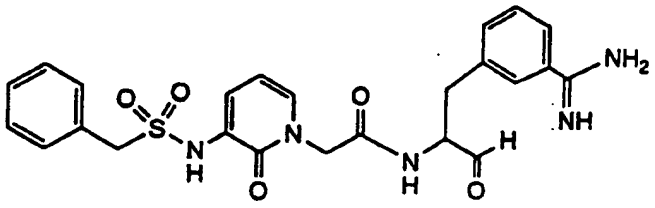
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-D,L-3-amidinophenyl alaninal semicarbazone



The compound of Example 80 (102 mg, 0.11 mmole) is treated with hydrofluoric acid/anisole (9:1) for 30 minutes at -20°C and 0°C for 30 minutes. After removal of the hydrofluoric acid, the resulting residue is dissolved in 20% aqueous acetic acid and washed with diethyl ether. The aqueous layer is lyophilized to a powder, then is purified by preparative HPLC (C-18, eluting with 10 to 40% acetonitrile-water gradient containing 0.1% trifluoroacetic acid) to give the title compound.

Example 82

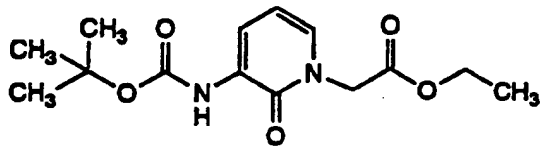
Preparation of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-D,L-3-amidinophenyl alaninal



The compound of Example 81 (16.6 mg, 30 micromole) is dissolved in methanol (1 mL) and 1% aqueous trifluoroacetic acid (5 mL), then formalin (0.23 mL) is added. After 40 minutes, the solution is filtered through a 2 micron filter, diluted to a volume of 15 mL with water, and then is purified by preparative HPLC (C-18, eluting with 10 to 40% acetonitrile-water gradient containing 0.1% trifluoroacetic acid). The fractions containing the title compound are pooled and lyophilized to give the title compound.

Example 83

Preparation of ethyl(3-[(N-t-butyloxycarbonyl)amino]-2-oxo-1,2-dihydropyridyl)acetate



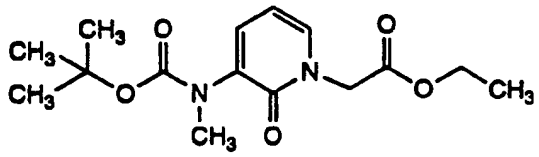
A stirred solution of the compound of Example 5 (44.5 g, 0.197 mole) in ethanol (200 mL) was hydrogenated over 10% Pd/C (2.25 g) for 16 hours under balloon pressure. Celite was added, and the reaction mixture was filtered through a pad of celite in a 600 mL fritted funnel (5 cm depth), using ethyl acetate to wash. The solvent was removed under vacuum, diluted with ethyl acetate (200 mL) and toluene (200 mL), and the solvent was removed under vacuum to give crude ethyl(3-amino-2-oxo-1,2-dihydropyridyl)acetate (40.0g, 0.204 mol) in quantitative yield.

A stirred solution of ethyl(3-amino-2-oxo-1,2-dihydropyridyl)acetate (2.00 g, 10 mmol) and sodium bicarbonate (1.69 g, 9.5 mmol) in 50% aqueous dioxane (20 ml) is cooled in an ice bath. A solution of di-t-butyldicarbonate (2.08 g, 20 mmol) in dioxane (10 ml) is added over a 5 minute period. After addition is complete, the solution is stirred for 16 hours at room temperature. The r action mixture is diluted with ethyl acetate (100

mL), washed with 1.0N HCl (until aqueous layer is pH 1), water, saturated sodium bicarbonate, and brine. The organic layer is dried over magnesium sulfate, and the solvent is removed. The title compound is isolated.

Example 84

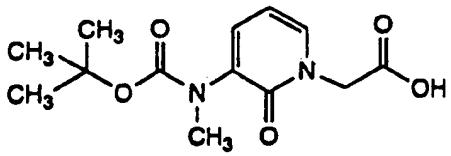
Preparation of ethyl(3-[(N-t-butyloxycarbonyl-N-methyl)amino]-2-oxo-1,2-dihydropyridyl)acetate



The compound of Example 83 (3.00 g, 10 mmol) and iodomethane (1.2 mL, 20 mmol) are dissolved in tetrahydrofuran (30 mL), and the solution is cooled to 0°C under a nitrogen atmosphere. Sodium hydride (0.44g of a 60% dispersion in mineral oil, 11 mmol) is added cautiously with gentle stirring. After the addition is complete, the reaction mixture is stirred at room temperature for 16 hours. Ethyl acetate (50 mL) is added, followed by water, to destroy the excess sodium hydride. The organic layer is washed with water, 5% aqueous sodium thiosulfate (to remove the iodine), water and brine, dried over magnsium sulfate, and evaporated. The title compound is isolated.

Example 85

Preparation of (3-[(N-t-butyloxycarbonyl-N-methyl)amino]-2-oxo-1,2-dihydropyridyl)acetic acid

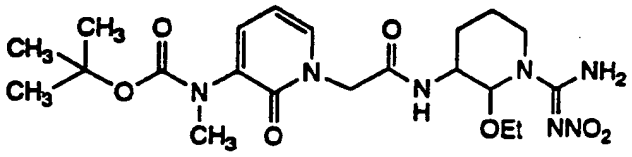


To a cooled (0°C) suspension of the compound of Example 84 (3.2 g, 10 mmole) in methanol (10 mL) is added

1.0N NaOH (12 ml) over a period of 10 minutes. After the addition is complete, the solution is allowed to warm to room temperature over a period of 3 hours. The solvent is reduced under vacuum, the residue is diluted with water (25 mL), and washed with ethyl acetate. The aqueous layer was acidified with 2.0N HCl to pH 1, extracted with ethyl acetate three times. The combined organic extracts are washed with water, then brine (twice). The solvent is removed, and the title compound is isolated.

Example 86

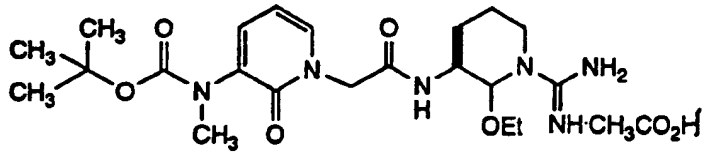
Preparation of [3-(N-t-butyloxycarbonyl-N-methyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-N^{g}-nitro-L-argininal ethyl cyclol



To a stirred suspension of the compound of Example 85 (2.2 g, 7.7 mmole), N^{g}-nitro-L-argininal ethyl cyclol, hydrochloride salt (2.47 g, 9.2 mmol), and N-hydroxybenzotriazole (1.17 g, 7.7 mmole) cooled to 0°C is added EDC (1.77g, 9.2 mmole). N-methylmorpholine (2.5 mL, 23 mmole) is added dropwise. After the addition is complete, the reaction is stirred at room temperature for 3 hours. The solvent is reduced under vacuum, and the resulting residue is dissolved in dichloromethane, washed with 2.0N HCl (to pH1), water, saturated sodium bicarbonate and brine. The extract is dried over magnesium sulfate, and the solvent is removed under vacuum. The title compound is isolated.

Example 87

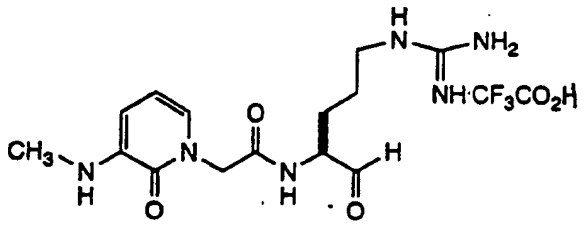
Preparation of [3-(N-t-butyloxycarbonyl-N-methyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ethyl cyclol, acetate salt



The compound of Example 86 (5.5 g, 11 mmole) in ethanol/acetic acid/water (4:1:1, 60 mL) is hydrogenated over 10% palladium on carbon (1.80 g) for 4 hours at 20 psi. Celite is added, and the solution is filtered through a 0.2 micron filter, washing the solid with ethanol/acetic acid/water (4:1:1, 60 mL). To the filtrate is added 10% palladium on carbon (1.80 g), and the solution is hydrogenated at 20-25 psi for 40 hours. Celite is added, and the solution is filtered through a 0.2 micron filter, washing the solid with water (200 mL). The solvent is reduced to a volume of 200 mL under reduced pressure, then washed with ethyl acetate (50 mL). The solvent from the aqueous layer is reduced to remove the volatiles, then the sample is lyophilized to yield the title compound.

Example 88

Preparation of [3-(N-t-butyloxycarbonyl-N-methyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, trifluoroacetate salt

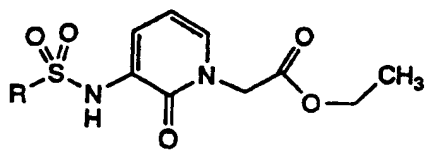


The compound of Exampl 87 (4.7 g, 9.2 mmole) is suspended in 3.0N HCl (100 mL) is added. After stirring for 3 hours, the reaction mixture is quenched with 2.5 M aqueous sodium acetate to pH 3.5 to 4, then filtered through a 2 micron filter. The filtrate is purified by preparative HPLC (Waters PrepPak cartridge, Delta-Pak C18, 300 angstrom column, 0-40% acetonitrile/water containing 0.1% trifluoroacetic acid). The clean fractions are combined to give the title compound.

Example 89

General Procedure for Reaction of Ethyl (3-amino-2-oxo-1,2-dihydropyridyl)acetate with sulfonyl or sulfamoyl chlorides

To a stirred solution of ethyl (3-amino-2-oxo-1,2-dihydropyridyl) acetate (5.89 g, 30 mmole) in dry tetrahydrofuran (300 mL) is added the 2,4,6-collidine (7.93 mL, 60 mmol) and the solution is cooled to 0°C under nitrogen. The appropriate sulfonyl or sulfamoyl chloride listed below (33 mmol) dissolved in tetrahydrofuran (25-75 mL) is added dropwise. After the addition is complete, the reaction mixture is stirred for 30 minutes to 1 hour at 0°C and then at ambient temperature for 0 to 72 hours. The reaction mixture is diluted with ethyl acetate, washed successively with 1.0N HCl, water, saturated sodium bicarbonate and brine, dried over magnesium sulfate, and the solvent is removed *in vacuo*. The residue is chromatographed on silica gel using a gradient system of dichloromethane and 1-4% methanol in dichloromethane to afford the product, judged pure by TLC (silica gel). Using this method and the starting materials listed below, intermediates having the formula given below are made:



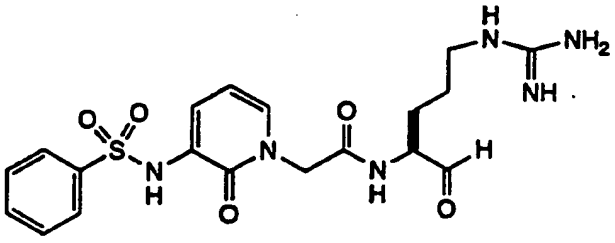
R=	Starting Material
phenyl	benzenesulfonyl chloride
1-naphthyl	1-naphthylsulfonyl chloride
2-naphthyl	2-naphthylsulfonyl chloride
2-carbomethoxyphenyl	2-carbomethoxybenzenesulfonyl chloride
2-trifluoromethylbenzyl	2-trifluoromethylbenzylsulfonyl chloride
2-cyclohexylamino	cyclohexylsulfamoyl chloride
2-trifluoromethylphenyl	2-trifluoromethylbenzenesulfonyl chloride
3-trifluoromethylphenyl	3-trifluoromethylbenzenesulfonyl chloride
4-trifluoromethylphenyl	4-trifluoromethylbenzenesulfonyl chloride
2-methylphenyl	2-methylbenzenesulfonyl chloride
3-methylphenyl	3-methylbenzenesulfonyl chloride
2-methyl-5-fluorophenyl	2-methyl-5-fluorobenzenesulfonyl chloride
2-methoxyphenyl	2-methoxybenzenesulfonyl chloride
3-methoxyphenyl	3-methoxybenzenesulfonyl chlorid

2-methoxy-5-chlorophenyl	2-methoxy-5-chlorobenzene-sulfonyl chloride
2-nitrophenyl	2-nitrobenzenesulfonyl chloride
2-trifluoromethoxyphenyl	2-trifluoromethoxybenzene-sulfonyl chloride
2,5-dichlorophenyl	2,5-dichlorobenzenesulfonyl chloride
2,5-dimethoxy	2,5-dimethoxybenzenesulfonyl chloride
2-fluorophenyl	2-fluorobenzenesulfonyl chloride
3-fluorophenyl	3-fluorobenzenesulfonyl chloride

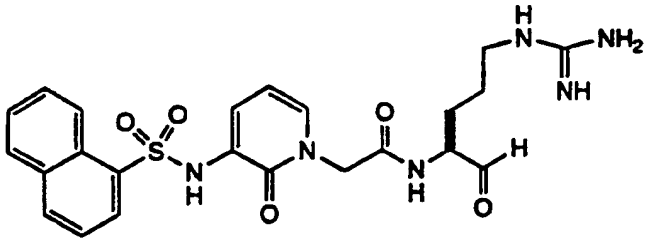
Example 90

General Procedure for the Preparation of Compounds of the Present Invention

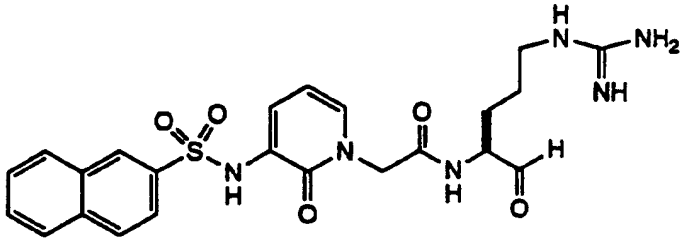
Following the four-step protocol outlined in Examples 7 to 10 (hydrolysis, coupling, hydrogenation and hydrolysis), certain of the intermediates of Example 89 are used to synthesize the following compounds of the present invention (as their trifluoroacetic acid salts):



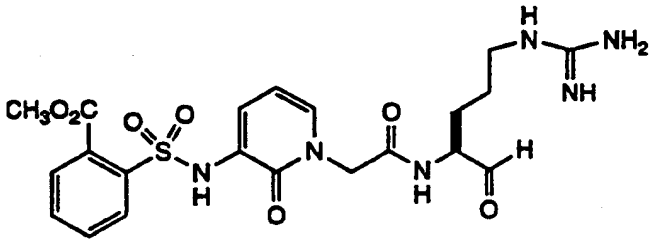
(3-phenylsulfonylamino-2-oxo-1,2-dihydro-pyridyl)acetyl-L-argininal (Compound B),



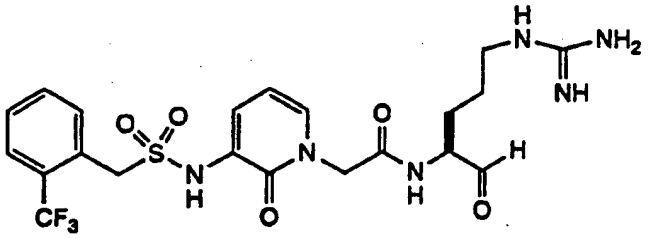
[3-(1-naphthyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



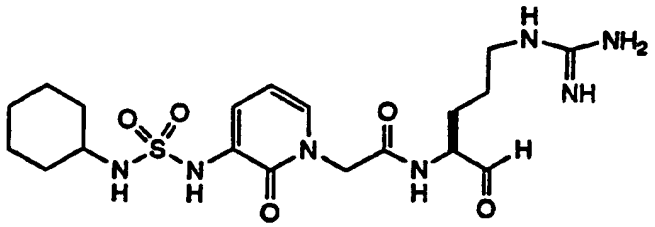
[3-(2-naphthyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



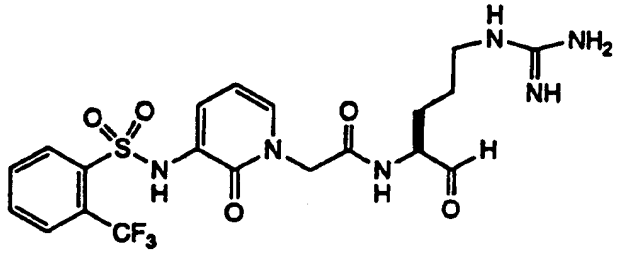
[3-(2-carbomethoxyphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



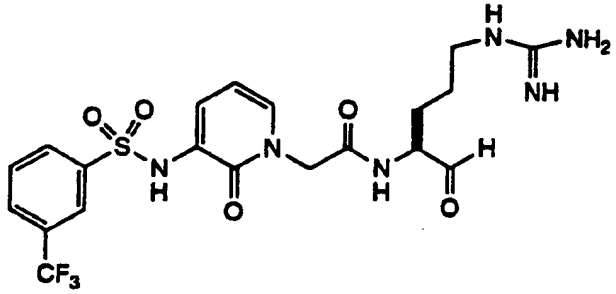
[3-(2-trifluoromethylbenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



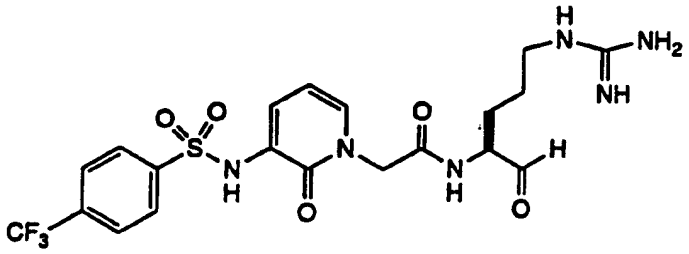
(3-cyclohexylaminosulfonylamino-2-oxo-1,2-dihydro-pyridyl)acetyl-L-argininal,



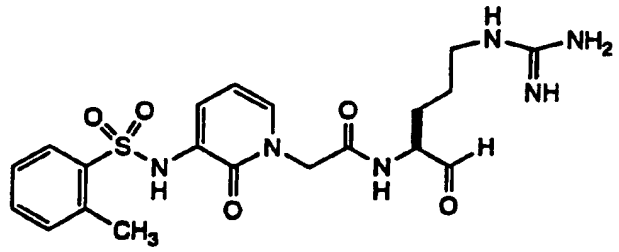
[3-(2-trifluoromethylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



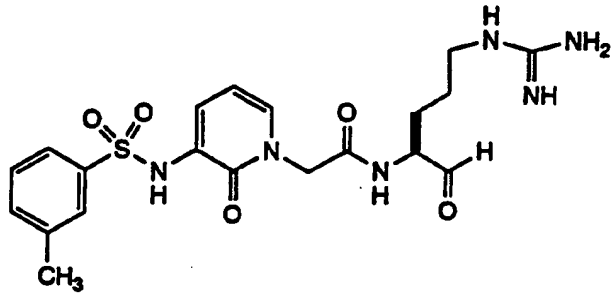
[3-(3-trifluoromethylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



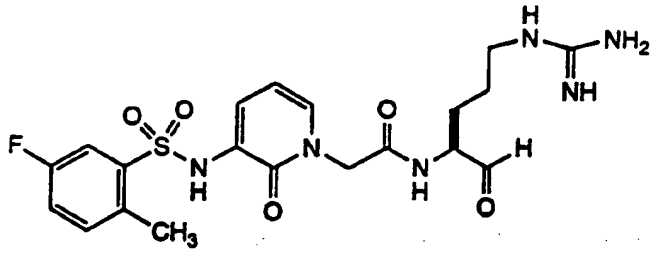
[3-(4-trifluoromethylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



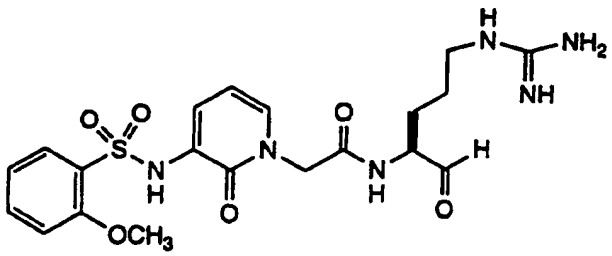
[3-(2-methylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



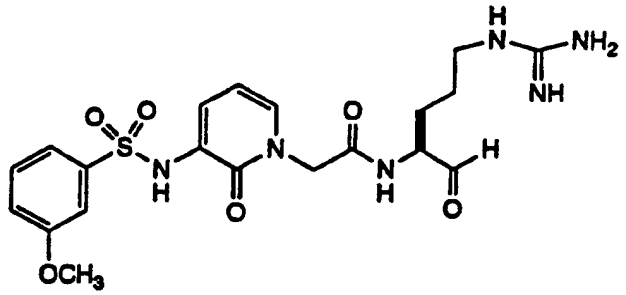
[3-(3-methylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



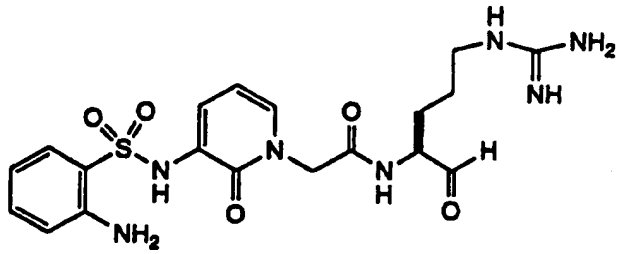
[3-(2-methyl-5-fluorophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



[3-(2-methoxylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal,



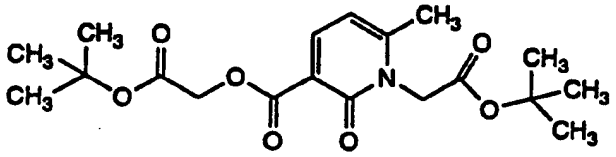
[3-(3-methoxylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal, and



[3-(2-aminophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal.

Example 91

Preparation of t-butyl [(t-butyl 3-carboxyacetate)-6-methyl-2-oxo-1,2-dihydro-1-pyridyl]acetate

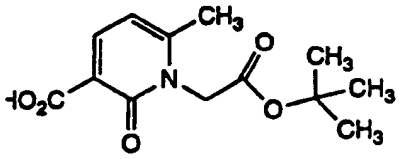


To a stirred solution of 2-hydroxy-6-methylpyridine-3-carboxylic acid (12.00 g, 78 mmole) in dimethylformamide (180 ml) was added potassium carbonate (22.8 g, 165 mmole) and t-butyl bromoacetate (24.2 mL, 165 mmole). After stirring for 36 hours, the reaction mixture was diluted with water (700 mL) and extracted with ethyl acetate (2X200 mL). The combined organic extracts were washed with brine and dri d over magnesium sulfate. The solvent was removed in vacuo. The residue was suction chromatographed through

flash silica gel using 10-50% ethyl acetate/hexanes to yield 22.46 g (75%) of the title compound as an oil. Rf = 0.10 (silica gel, 33% ethyl acetate/hexanes).

Example 92

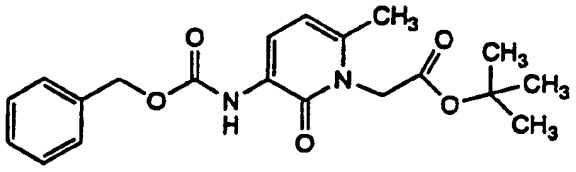
Preparation of t-butyl (3-carboxy-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetate



To a stirred solution of the compound of Example 91 (22.46 g, 59 mmole) in tetrahydrofuran (270 mL) was added 1.0M lithium hydroxide (90 mL, 90 mmole). After 2 hours, the solution was concentrated. The solution was diluted with water (150 mL) and extracted with diethyl ether. The aqueous layer was acidified to pH 3 with 1M sodium bisulfate, and extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over magnesium sulfate and the solvent was removed in vacuo. The title compound was isolated (18.02 g) in quantitative yield.

Example 93

Preparation of t-butyl (3-benzyloxycarbonylamino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetate

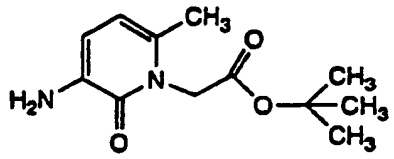


To the compound of Example 92 (13.0 g, 48.7 mmole) suspended in dioxane (150 ml), was added triethylamine (7.7 mL, 55 mmole) dropwise rapidly with stirring followed by diphenylphosphoryl azide (16 mL, 73 mmole). The suspension

was heated f r 2 hours using a preheat d 110°C il bath. Benzyl alcohol (7.6 g, 73 mmole) was then added and the mixture was stirred at 110°C for 20 hours. The reaction mixture was cooled and concentrated. The residue was suspended in ethyl acetate (400 mL) and was washed with 3% HCl, then brine, dried over magnesium sulfate and concentrated. The crude product was chromatographed on flash silica gel using 20-67% ethyl acetate/hexanes to afford the title compound (14.2g, 78% yield) as a white solid. Rf = 0.53 (silica gel, 33% ethyl acetate/hexanes).

Example 94

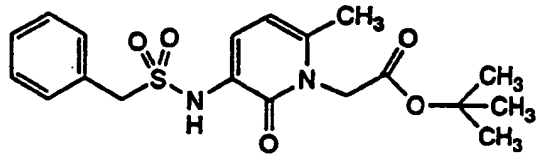
Preparation of t-butyl (3-amino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetate



Using similar procedures to that described in Example 106 herein, the title compound (0.89 g) was prepared from the compound of Example 93 (1.7 g) in 82% yield. Rf = 0.69 (silica gel, 10% methanol/dichloromethane).

Example 95

Preparation of t-butyl (3-benzylsufonylamino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetate

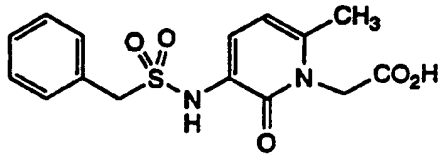


Collidine (0.59 mL, 4.5 mmole) was added in one portion to a stirred solution of the compound of Example 94 (0.89 g, 3.7 mm le) and benzylsulfonyl chloride (0.86g, 4.5 mmole) in acetonitrile (20 ml) cooled in an ice bath. The solution was stirred for 5 minutes at 0°C, followed by 45 minutes at

room temperature. Th reaction mixture was quenched with water, then diluted with ethyl acetate (100 mL), washed with 3% HCl (until aqueous layer was pH 1), and brine, dried over magnesium sulfate, and the solvent was removed. The residue was dissolved in methanol, concentrated to a volume of approximately 3 mL, and the product was precipitated with the addition of diethyl ether. The precipitate was filtered to give 0.67 g of the title compound. The filtrate was concentrated and chromatographed on flash silica gel using 20 to 67% ethyl acetate hexanes as eluent. An additional 0.20 g of the title compound was recovered. A total of 0.87 g of the title compound (59% yield) was recovered. Rf = 0.29 (silica gel, 33% ethyl acetate/hexanes).

Example 96

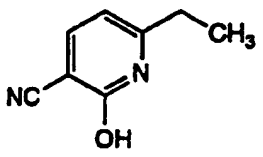
Preparation of (3-benzylsulfonylamino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetic acid



To a cooled (0°C) solution of the compound of example 95 (0.87 g, 2.2 mmole) in dichloromethane (10 mL) was added trifluoroacetic acid (10 mL). After stirring for 30 minutes, the ice bath was removed and the solution stirred for 2.5 hours at room temperature. The reaction mixture was concentrated. The resulting solid was triturated with diethyl ether (15 mL) and dried *in vacuo*. The title compound (0.73 g) was isolated in 98% yield. Rf = 0.13 (silica gel, 10% methanol/dichloromethane).

Example 97

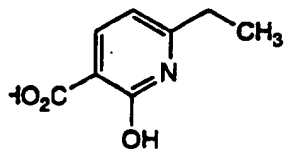
Preparation of 2-hydroxy-6-ethylpyridine-3-carbonitrile



To a suspension of 1-hydroxy-6-methylpyridine-3-carbonitrile (12.24 g, 0.091 mole) in tetrahydrofuran (100 mL) cooled to -78°C under a nitrogen atmosphere was added dropwise lithium diisopropylamide (100 mL of a 2.0M solution in heptane/tetrahydrofuran/ethylbenzene, 0.20 mole). After the addition was complete, the solution was stirred in an ice bath for 2 hours. Iodomethane (6.25 mL, 0.10 mol) was added, and the reaction mixture was stirred for an additional 2.5 hours at 0°C and 30 minutes at room temperature. Water (300 mL) and 1.0N NaOH 50 mL) were added. The aqueous solution was washed with ethyl acetate (150 mL), acidified with 1.0N sodium bisulfate to pH 4, and extracted with 10% methanol/ethyl acetate twice (500 mL total). Sodium chloride was added to the aqueous layer, and the solution was extracted with 10% isopropanol/ethyl acetate twice (500 mL total). The combined organic layers were washed with brine, dried over magnesium sulfate and the solvent removed under reduced pressure. The residue was recrystallized from methanol/isopropanol to give the title compound (7.56 g) as orange needles in 56% yield. Rf = 0.26 (silica gel, 10% isopropanol/chloroform); m.p. 235 to 240°C (decomp).

Example 98

Preparation of 2-hydroxy-6-ethylpyridine-3-carboxylic acid

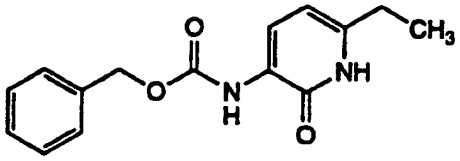


The compound of Example 97 (7.56 g, 51 mmole) was refluxed in 50% sulfuric acid (50 mL) for 3 hours. The reaction

mixture was cooled, and poured into water (250 mL). The solution was allowed to stand at 4°C for 16 hours. The solid was filtered, washed with water and air dried to afford the title compound (6.03 g) in 71% yield as a tan solid; m.p. 190.5 to 193°C.

Example 99

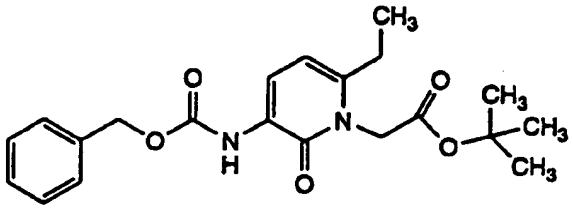
Preparation of 3-benzyloxycarbonylamino-6-ethyl-2-oxo-1,2-dihydro-1-pyridine



Using similar procedures to that described above in Example 93, the title compound (0.74 g) was prepared from the compound of Example 98 (1.00 g) in 45% yield. Rf = 0.18 (silica gel, 20% ethyl acetate/dichloromethane); m.p. 153.5 to 154°C.

Example 100

Preparation of t-butyl (3-benzyloxycarbonylamino-6-ethyl-2-oxo-1,2-dihydro-1-pyridyl)acetate



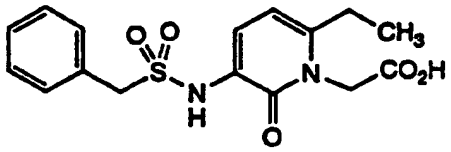
To a solution of the compound of Example 99 (150 mg, 0.55 mmole) in tetrahydrofuran (2 mL) was added lithium hexamethyldisilazide (0.61 mL of a 1.0 M solution in tetrahydrofuran, 0.61 mmole). After 1.5 hours, t-butyl bromoacetate (0.089 mL, 0.61 mmole) was added. The reaction mixture was stirred for 16 hours, then diluted with water (5 mL) and saturated ammonium chloride (5 mL), and extracted

with ethyl acetate (3X5 mL). The combined organic layers were washed with brine, dried over magnesium sulfate and the solvent was reduced. Hexanes (10 mL) were added, and the solvent was removed *in vacuo* to afford 0.20 g of the title compound as a white solid in 94% yield. Rf = 0.76 (silica gel, 20% ethyl acetate/dichloromethane).

Example 101

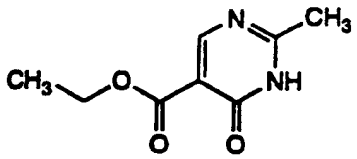
Preparation of (3-benzylsulfonylamino-6-ethyl-2-oxo-1,2-dihydro-1-pyridyl)acetic acid

Following the three step protocol outlined in Examples 94-96, the intermediate of Example 100 is used to synthesize the following compound of the present invention.



Example 102

Ethyl 2-methyl-pyrimidin-6(1H)-one-5-carboxylate

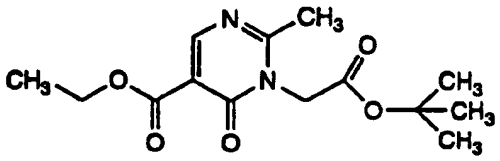


Acetamidine acetate (37.21 g, 0.31 mole) and diethyl ethoxymethylenemalonate (63 mL, 0.31 mole) were refluxed for 4h in ethanol (60 mL). The reaction mixture was allowed to cool for 15 minutes, then acetamidine acetate (37.21 g, 0.31 mole) was added. The reaction mixture was refluxed for 22 hours, allowed to cool to room temperature, and diluted with water (200 mL) and dichloromethane (200 mL). The aqueous layer was extracted with 10% isopropanol/dichloromethane (2X200 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL), dried over magnesium sulfate,

filtered and the solvent was removed. The residue was recrystallized from chloroform/hexanes in two crops to afford the title compound (24.92 g) in 46% yield as yellowish crystals. R_f = 0.27 (silica gel, 10% isopropanol in dichloromethane); m.p. 187 to 188°C.

Example 103

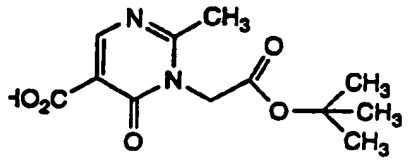
Preparation of Ethyl 3-(t-butyl acetyl)-2-methyl-pyrimidin-6(1H)-one-5-carboxylate



Tetra-n-butylammonium fluoride(27.4 mL of a 1.0 M solution in tetrahydrofuran, 27.4 mmole) was diluted with hexanes (30 mL), the solvent was removed under reduced pressure, and the white crystals were taken up in dimethoxyethane (50 mL). t-Butyl bromoacetate (3.0 mL, 20.1 mmole) was added while stirring, followed by the compound of Example 102 (2.50 g, 13.7 mmole). The mixture was stirred under a nitrogen atrmospherre for 1.5 hours at room temperature. The reaction mixture was diluted with water (100 mL), and extracted with ethyl acetate (3X50 mL). The combined organic extracts were washed with water, then brine (20 mL each), dried over magnesium sulfate, and the solvent was removed. The residue was purified through flash silica gel using 50% ethyl acetate, then ethyl acetate as eluent. The title compound was isolated to yield 1.81 g (45%). R_f = 0.24 (silica gel, 20% ethyl acetate in dichloromethane).

Example 104

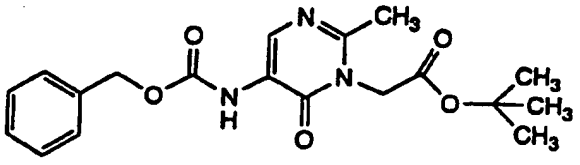
Preparation of 3-(t-butyl acetyl)-2-methyl-pyrimidin-6(1H)-one-5-carboxylic acid



To the compound of Example 103 (10.16 g, 0.034 mole), suspended in methanol (70 ml) and cooled in an ice bath, 1.0N lithium hydroxide (38 mL, 0.038 mole) was added dropwise rapidly with stirring. The ice bath was removed. After 2h, reaction mixture was neutralized to pH 7 with 1.0N hydrochloric acid. The solvent was reduced under vacuum, the residue diluted with water (50 mL) and washed with ethyl acetate (2X25 mL) The aqueous layer was acidified with 2.3N HCl to pH 1, extracted with ethyl acetate (50 mL), followed by dichloromethane twice (30 mL total). The combined organic extracts are washed with brine (3X10 mL). The solvent was dried over magnsium sulfate, and removed *in vacuo*. The residue was recrystallized from ethyl acetate/diethyl ether (first crop) and ethyl acetate/diethyl ether/hexanes (second crop) to afford 4.37 g (48%) of the title compound. R_f = 0.31 (silica gel, 1% acetic acid/10% isopropanol in chloroform).

Example 105

Preparation of t-butyl 2-methyl-5-benzyloxycarbonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetate

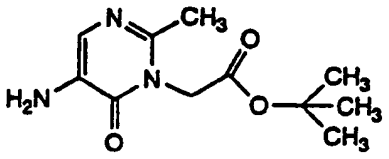


To the compound of Example 104 (4.20 g, 0.0157 mole) suspended in dioxane (50 ml), was added triethylamine (4.4 mL, 0.0313 mole) dropwise rapidly with stirring followed by diphenylphosphoryl azide (3.7 mL, 0.0172 mole). The

suspension was heated for 2 hours using a preheated 100°C oil bath. Benzyl alcohol (3.2 g, 0.0313 mole) was then added and the mixture was stirred at 100°C overnight. The reaction mixture was cooled and concentrated. The residue was suspended in ethyl acetate (100 mL) and was washed with saturated ammonium chloride, 1.0N NaOH , water (twice) and brine. The extract was dried over magnesium sulfate and concentrated. The crude product was chromatographed on flash silica gel using 10 to 25% ethyl acetate/dichloromethane to give the title compound (3.07 g, 53% yield) as a light yellow solid. R_f = 0.24 (silica gel, 20% ethyl acetate in dichloromethane).

Example 106

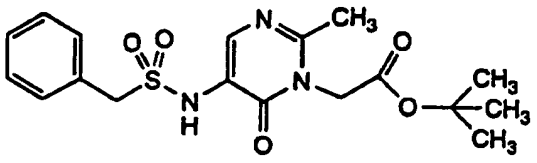
Preparation of t-butyl 2-methyl-5-amino-6-oxo-1,6-dihydro-1-pyrimidinylacetate



The compound of Example 105 (1.50 g, 4.0 mmol) was hydrogenated over 10% palladium on carbon (0.16 g) in ethanol (30 mL) at balloon pressure overnight. Celite was added, and the solution was filtered. The solvent was reduced. Hexanes were added and the solvent was removed *in vacuo* to afford 0.97 g (quantitative yield) of the title compound as a white solid.
R_f = 0.24 (silica gel, 10% isopropanol in chloroform).

Example 107

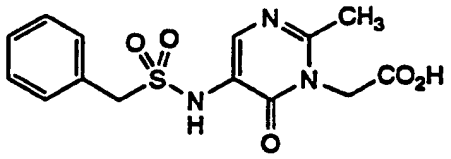
Preparation of t-butyl 2-methyl-5-benzylcarbonyloxyamino-6-oxo-1,6-dihydro-1-pyrimidinylacetate



Benzylsulfonyl chloride (1.06 g, 5.6 mmole) was added in one portion to a stirred solution of the compound of example 106 (0.89 g, 3.7 mmole) and 4-methylmorpholine (1.47 mL, 11.2 mmole) in tetrahydrofuran (10 ml). The solution was stirred for 2 hours. The reaction mixture was concentrated, then diluted with ethyl acetate (100 mL), washed with 1.0N HCl (until aqueous layer is pH 1), water, saturated sodium bicarbonate and brine. The organic layer was dried over magnesium sulfate, and the solvent was removed. The residue was recrystallized from ethyl acetate (first crop) and ethyl acetate/ether/hexanes (second crop). The second crop was treated with 1.0 M potassium carbonate (3 mL) and methanol(10 mL) for 2 hours. The solution became homogeneous. The reaction mixture was acidified to pH 7 with 1.0N HCl. The solvent was reduced, and the aqueous solution was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried over magnesium sulfate and the solvent was removed *in vacuo*. In a similar fashion the first crop was treated with 1.0M potassium carbonate and methanol. A total of 1.20 g of the title compound (82% yield) was recovered as a white solid. R_f = 0.26 (silica gel, 20% ethyl acetate in dichloromethane).

Example 108

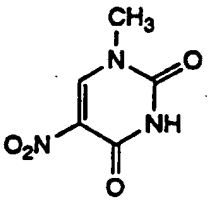
Preparation of 2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetic acid



To the compound of Example 107 (1.15 g, 2.9 mmole) was treated with 50% trifluoroacetic acid/dichloromethane (10 mL). After 1 hour, the reaction mixture was concentrated, then diluted with diethyl ether (100 mL). The solution was allowed to stand overnight, then the solvent was removed under reduced pressure. The residue was partitioned between saturated sodium bicarbonate (25 mL) and ethyl acetate (10 mL). The aqueous layer was washed with ethyl acetate, then acidified with 2.3M HCl to pH 1. The preciptiate was filtered, washed with water and dried under vacuum to give the title compound (0.75 g, 76% yield) as a white solid. m.p. 244 to 246°C (decomp.).

Example 109

Preparation of 5-nitro-1-methyl-uracil

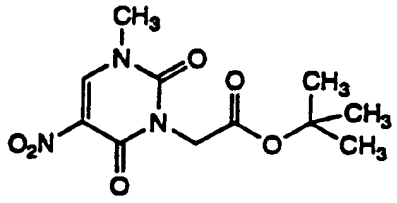


5-nitrouracil (10.00 g, 64 mmole) and potassium carbonate were stirred in dimethylformamide (50 mL) for 15 min. A solid formed. Iodomethane (5.3 mL, 85 mmol) was added and the flask was shaken until the solid dissolved. Aft r the reaction mixture was stirred for 30 min., 2% NaOH (w/v) (200 mL) was added, followed by water (100 mL). The solution was washed with ethyl acetate (100 mL), and the aque us layer was acidified to pH 3 with 1.0N HCl. A

precipitate formed as the pH was lowered. After allowing the heterogeneous solution to stand for 16 hours, the product was filtered, washed with water, and air dried. The title compound was isolated in 77% yield as a yellow powder; m.p. 249 to 250°C.

Example 110

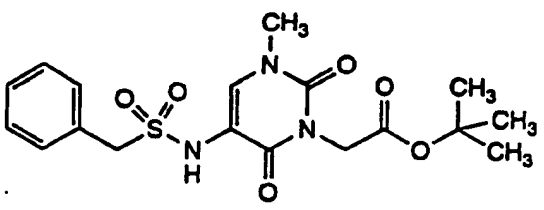
Preparation of t-butyl (5-nitro-1-methyl-uracilyl)acetate



Sodium hydride (0.51 g of a 60% dispersion in mineral oil, 13 mmol) was washed with pentane three times (4 mL each). The compound of Example 109 (2.00 g, 12 mmole) was added portionwise. After the addition was complete, the reaction mixture was stirred for 30 minutes under a nitrogen atmosphere. t-Butyl bromoacetate (1.73 g, 12 mmole) was added in one portion, and the solution was stirred for 3 hours. The reaction mixture was diluted with water (200 mL), and extracted with ethyl acetate (3X50 mL). The combined organic extracts were washed with water (3X50 mL), then brine, dried over magnesium sulfate. The solvent was concentrated, hexanes were added and the solvent was removed *in vacuo* to afford the title compound (1.97 g) in 59% yield. Rf = 0.38 (silica gel, 20% ethyl acetate in dichloromethane.

Example 111

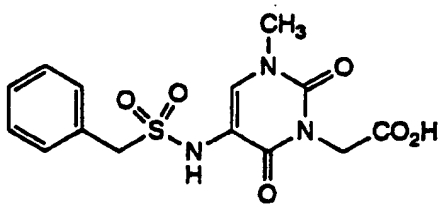
Preparation of Ethyl 5-(benzylsulfonylamino-1-methyl-uracilyl)acetate



Using similar procedures to that described above in Example 6, but employing 3 equivalents of 4-methyl morpholine as base during the reaction with benzylsulfonyl chloride, the title compound was prepared from the compound of Example 110 in 48% yield; m.p. 165 to 166°C.

Example 112

Preparation of 5-(benzylsulfonylamino-1-methyl-uracilyl)acetic acid

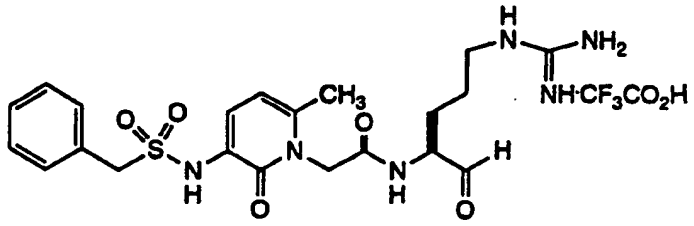


Using a similar procedure to that described above in Example 108, the title compound was prepared from the compound of Example 111 in 88% yield; m.p. 200 to 201°C.

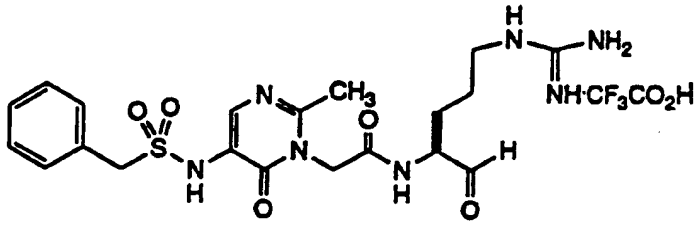
Example 113

General Procedure for Preparation of Compounds of the Present Invention

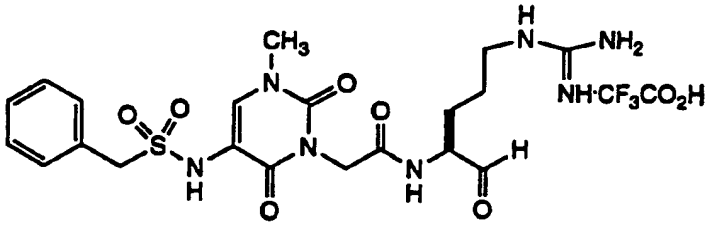
Following the three-step protocol outlined in Examples 8 to 10 (coupling, hydrogenation, hydrolysis) the intermediates of Examples 96, 108 and 112 were used to synthesize the following compounds of the present invention:



(3-benzylsulfonylamino-6-methyl-2-oxo-1,2-dihydro-1-pyridyl)acetyl-L-argininal, trifluoroacetate salt (Compound C);



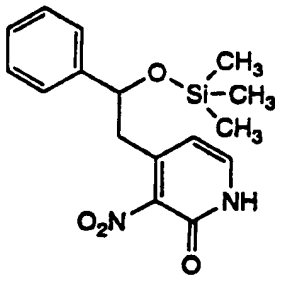
(5-benzylsulfonylamino-2-methyl-6-oxo-1,6-dihydro-1-pyrimidinyl)acetyl-L-argininal, trifluoroacetate salt (Compound D); and



(5-benzylsulfonylamino-1-methyl-uracilyl)acetyl-L-argininal, trifluoroacetate salt (Compound E).

Example 114

Preparation of 4-(2-trimethylsiloxyphenethyl)-3-nitro-2-oxo-1,2-dihydro-1-pyridine

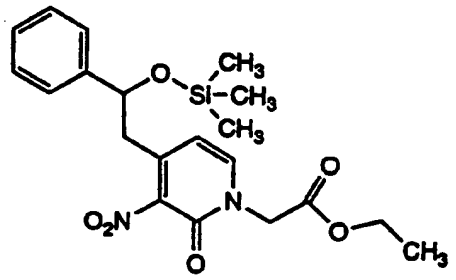


A suspension of 4-methyl-3-nitro-2-pyridone (3.08 g, 20 mmole) in tetrahydrofuran (50 mL) was cooled to 0°C. Lithium hexamethyldisilazide (21 mL of a 1M solution in tetrahydrofuran, 21 mmole) was added to the reaction over 15 minutes. After stirring for 45 minutes, trimethylsilyl chloride (2.7 mL, 21 mmole) was added. After an hour, another portion of lithium hexamethyldisilazide (21 mL of a 1M solution in tetrahydrofuran, 21 mmole) was added to the solution. After 30 minutes, freshly distilled

benzaldehyde (2.1 mL, 21 mmole) was added. The reaction was allowed to warm to room temperature and after 18 hours, it was quenched with aqueous ammonium chloride (20 ml), extracted with ethyl acetate (150 ml), washed with brine (50 ml), and dried over magnesium sulfate. The product was purified by chromatography with silica gel, eluting with 2-10% methanol/dichloromethane. Recrystallization from toluene yielded 0.79 g (8.5%) of the title compound. R_f = 0.25 (silica gel, 50% ethyl acetate/hexanes).

Example 115

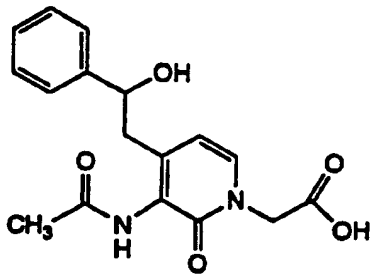
Preparation of Ethyl [4-(2-trimethylsiloxyphenethyl)-3-nitro-2-oxo-1,2-dihydro-1-pyridyl]acetate



To a solution of the compound of Example 114 (0.79 g, 2.4 mmole) in tetrahydrofuran at 0°C was added lithium hexamethyldisilazide (2.5 mL of a 1M in tetrahydrofuran. 2.5 mmole) over 5 minutes. After 30 minutes, ethyl bromoacetate (0.28 mL, 2.5 mmole) was added. The reaction was allowed to warm to room temperature and after 8 hours, it was quenched with aqueous ammonium chloride (5 mL), extracted with ethyl acetate (75 mL), washed with brine (30 mL), and dried over magnesium sulfate. The product was purified by chromotography with silica gel, eluting with (33%) ethyl acetate/hexanes to yield 0.76 g of the title compound (81% yield). R_f = 0.45 (silica gel, 50% ethyl acetate/hexanes).

Example 116

Preparation of [3-acetamido-4-(2-hydroxyphenethyl)-2-oxo-1,2-dihydro-1-pyridyl]acetic acid

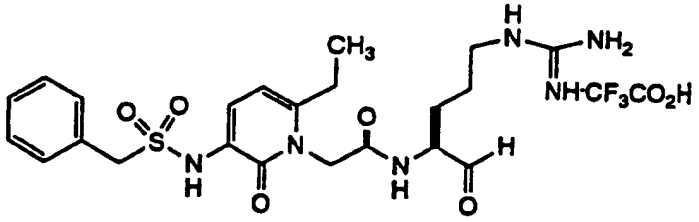


The compound of Example 115 (760 mg, 1.82 mmole) was dissolved in ethyl acetate (10 mL). Acetic anhydride (0.69 mL, 7.3 mmole) and 10% palladium on carbon (75 mg) were added and the reaction was stirred under a hydrogen balloon for 18 hours. The reaction was then filtered through celite and concentrated. The residue was dissolved in tetrahydrofuran (7.0 mL) and 1.0M lithium hydroxide was added (3.6 mL, 3.6 mmole). The reaction was stirred for 22 hours at which time additional 1.0M lithium hydroxide (2.0 mL, 2.0 mmole) and methanol (2.0 mL) were added. The reaction was stirred for 48 hours at room temperature, then was diluted with water (20 mL) and washed with ethyl acetate (20 mL). The aqueous layer was acidified with concentrated HCl to pH~3, and the product was extracted into ethyl acetate (50 mL), washed with brine (40 mL), and dried over magnesium sulfate. Back extraction of the aqueous layer with 30% isopropanol/dichloromethane yielded a total of 440 mg (96%) of the title compound. ^{1}H NMR (CD_3OD): delta 2.15 (3H, s), 2.81-2.94 (2H, m), 4.70 (2H, s), 4.9 (1H, dd, J=5.3, 8.2 Hz), 6.3 (1H, d, J=7.1 Hz), 7.22-7.36 (5H, m), 1.73 (1H, d, J=7.1 Hz).

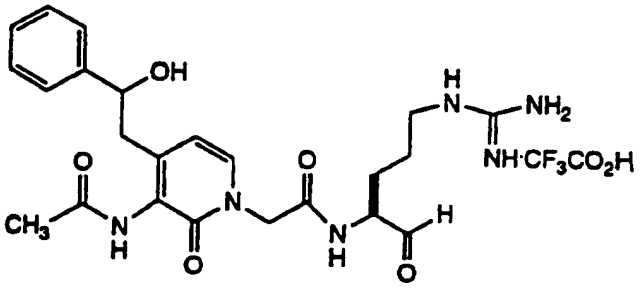
Example 117

General Procedure for Preparation of Compounds of the Present Invention

Following the three-step protocol outlined in Examples 8 to 10 (coupling, hydrogenation, hydrolysis) the intermediates of Examples 101 and 116 are used to synthesize the following compounds of the pr sent invention:



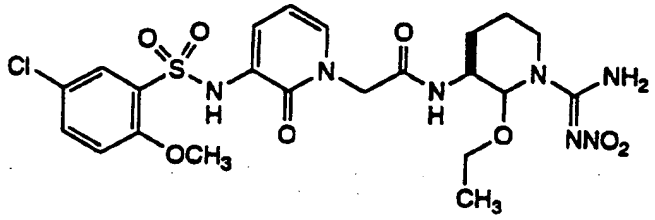
(3-benzylsulfonylamino-6-ethyl-2-oxo-1,2-dihydro-1-pyridyl)acetyl-L-argininal, trifluoroacetate salt, and



[3-acetamido-4-(2-hydroxyphenethyl)-2-oxo-1,2-dihydro-1-pyridyl]acetyl-L-argininal, trifluoroacetate salt.

Example 118

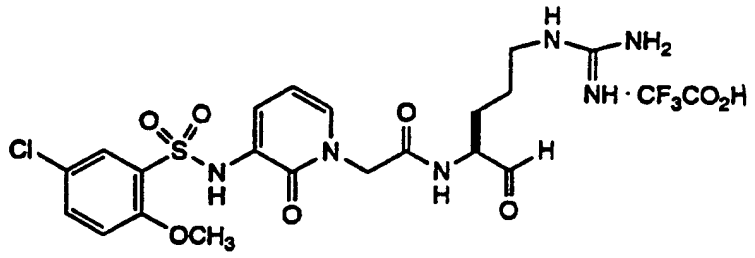
(5-chloro-2-methoxy-phenylsulfonyl-3-amino-2-oxo-1,2-dihydropyridyl)acetyl-L-N^g-nitro-argininal-ethyl cyclol



Following the two-step protocol outlined in Examples 7 to 8 (hydrolysis, coupling), the title compound is prepared from an intermediate of Example 89, ethyl (2-methoxy-5-chloro-benzenesulfonyl-3-amino-2-oxo-1,2-dihydropyridyl)acetate.

Example 119

Preparation of (5-chloro-2-methoxy-phenylsulfonyl-3-amino-2-oxo-1,2-dihydropyridyl)acetyl-L-Argininal, trifluoroacetate salt

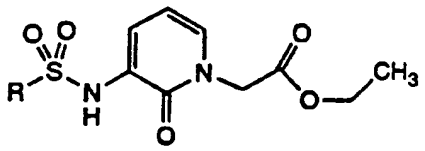


To a stirred solution of the compound of Example 118 (280 mg, 0.47 mmol) in ethyl alcohol (5 mL), a mixture of freshly made 20% titanium(III) chloride solution in water (3.7 mL, 4.7 mmol) and 4.0M ammonium acetate buffer, pH 5.0 (7.4 mL) was added. The reaction mixture was stirred at room temperature. After the reaction was complete (30-45 min.), the excess of titanium(III) chloride was oxidized by bubbling the air through the reaction mixture (30 min.). The solvent was removed *in vacuo*. The residual was taken into water (50 mL) and then centrifuged at 3,000 rpm for 10 minutes. The supernatant was decanted, and the solid was washed with water (30 mL) and centrifuged. The combined supernatants were concentrated to 25 mL. The solution was cooled down to 0°C with ice bath. 12N hydrochloric acid (25 mL) was added, and the ice bath was removed. The reaction mixture was stirred at room temperature. After the reaction was complete (30-45 minutes), the reaction mixture was quenched with water (150 mL) and sodium acetate (40 g), and then filtered. The aqueous solution was purified by reverse phase HPLC with C-18 column using a gradient system of 17 to 35% acetonitrile in water with 0.1% of trifluoroacetic acid over 30 min. to afford 160 mg of the title compound (160 mg, 0.31 mmol). MS: 513 ($M+H^+$).

Example 120

General Procedure for Reaction of Ethyl (3-amino-2-oxo-1,2-dihydropyridyl)acetate with sufonyl chlorides

To a stirred solution of ethyl (3-amino-2-oxo-1,2-dihydropyridyl) acetate (5.89 g, 30 mmole) in dry tetrahydrofuran (300 mL) is added 2,4,6-collidine (7.93 mL, 60 mmol) and the solution is cooled to 0°C under nitrogen. The appropriate sulfonyl or sulfamoyl chloride listed below (33 mmol) dissolved in tetrahydrofuran (25 to 75 mL) is added dropwise. After the addition is complete, the reaction mixture is stirred for 30 minutes to 1 hour at 0°C and then at ambient temperature for 0 to 72 hours. The reaction mixture is diluted with ethyl acetate, washed successively with 1.0N HCl, water, saturated sodium bicarbonate and brine, dried over magnesium sulfate, and the solvent removed in vacuo. The residue is chromatographed on silica gel using a gradient system of dichloromethane and 1 to 4% methanol in dichloromethane to afford the product, judged pure by TLC (silica gel). Using this method and the starting materials listed below, intermediates having the formula given below are made:



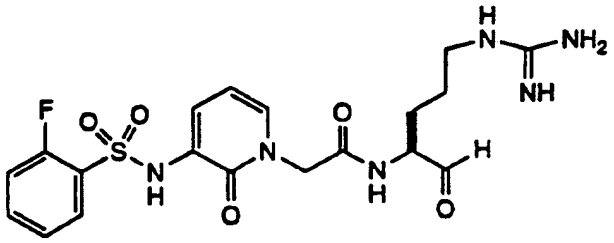
R=	Starting material
2-fluorophenyl	2-fluorobenzenesulfonyl chloride
3-fluorophenyl	3-fluorobenzenesulfonyl chloride
2-trifluoromethoxyphenyl	2-trifluoromethoxybenzene-sulfonyl chloride
2,5-dimethylphenyl	2,5-dimethylbenzenesulfonyl chloride

2,5-dimethoxyphenyl	2,5-dimethoxybenzenesulfonyl chloride
2,6-difluorophenyl	2,6-difluorobenzenesulfonyl chloride
phenethyl	2-phenylethanesulfonyl chloride
cyclohexylmethyl	cyclohexylmethanesulfonyl chloride
2,5-dichlorophenyl	2,5-dichlorobenzenesulfonyl chloride
2-fluorobenzyl	(2-fluorophenyl)methanesulfonyl chloride
3-fluorobenzyl	(3-fluorophenyl)methanesulfonyl chloride
3-trifluoromethylbenzyl	(3-trifluoromethyl-phenyl)methanesulfonyl chloride
2-carbomethoxybenzyl	(2-carbomethoxyphenyl)methane-sulfonyl chloride
3-carbomethoxybenzyl	(3-carbomethoxyphenyl)methane-sulfonyl chloride
2,6-difluorobenzyl	(2,6-difluorophenyl)methane-sulfonyl chloride
2,5-difluorobenzyl	(2,5-difluorophenyl)methane-sulfonyl chloride
2,4-difluorobenzyl	(2,4-difluorophenyl)methane-sulfonyl chloride

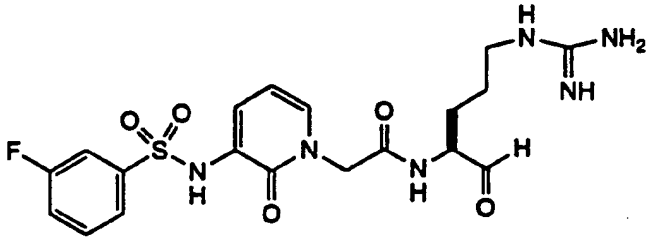
Example 121

General Procedure for the Preparation of Compounds of the Present Invention

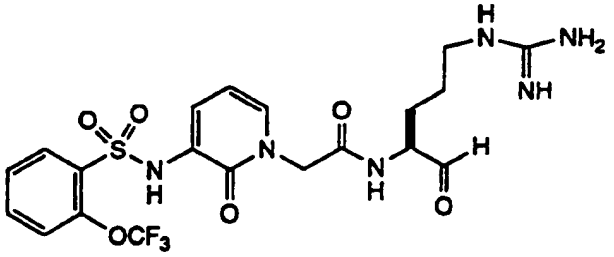
Following the four-step protocol outlined in Examples 7 to 10 (hydrolysis, coupling, hydrogenation and hydrolysis), the intermediates of Example 120 are used to synthesize the following compounds of the present invention (as their trifluoroacetic acid salts):



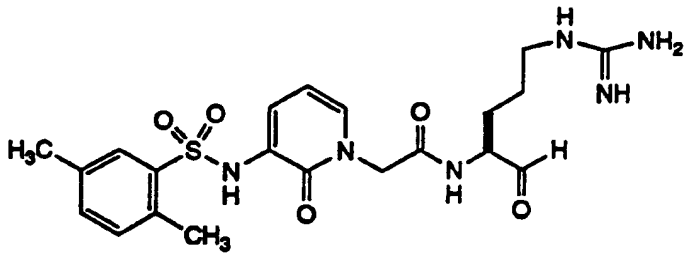
3-(2-fluorophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121A);



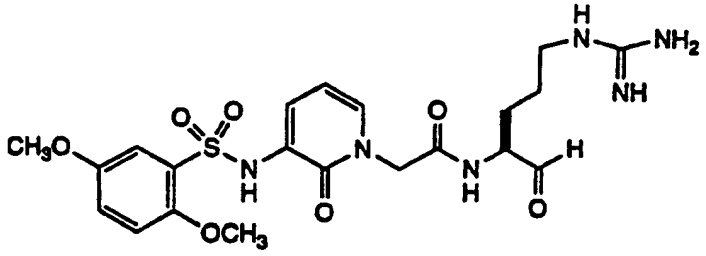
3-(3-fluorophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121B);



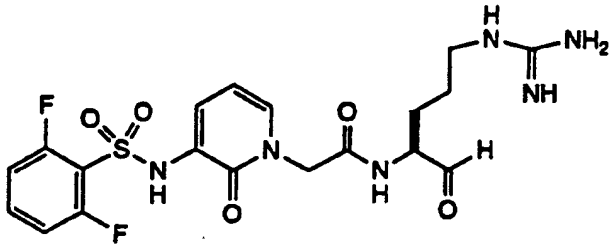
(3-(2-trifluoromethoxyphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl)acetyl-L-argininal (Compound 121C);



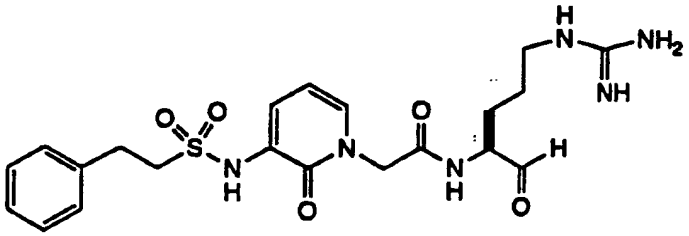
[3-(2,5-dimethylphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121D);



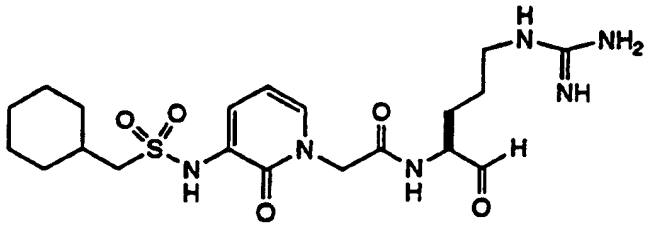
[3-(2,5-dimethoxyphenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121E);



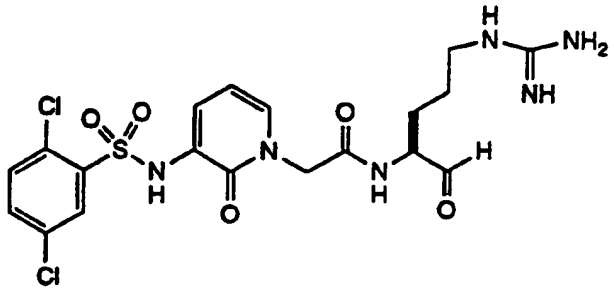
[3-(2,6-difluorophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121F);



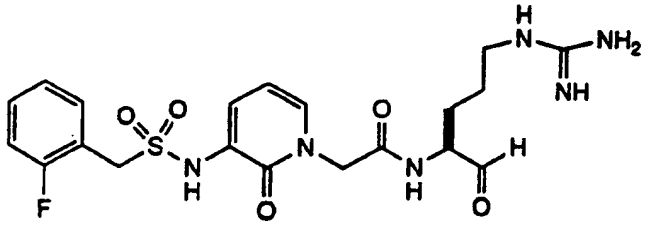
[3-(phenethyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121G);



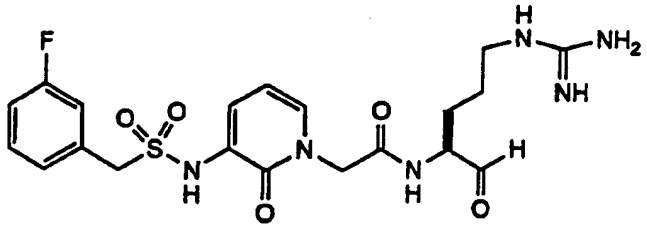
(3-cyclohexylmethylsulfonylamino-2-oxo-1,2-dihydro-pyridyl)acetyl-L-argininal (Compound 121H);



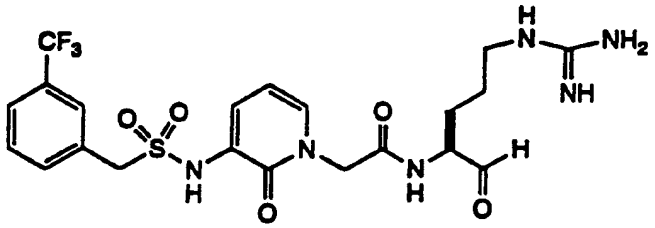
[3-(2,5-dichlorophenyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121I);



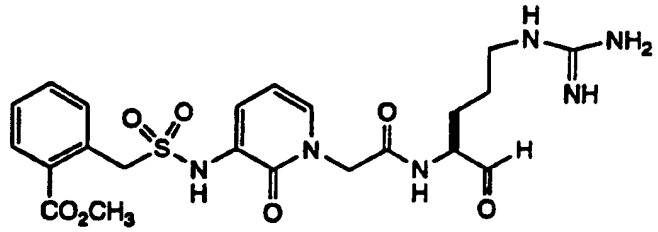
3-(2-fluorobenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121J);



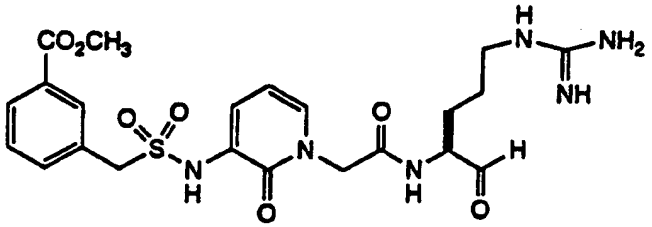
3-(3-fluorobenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121K);



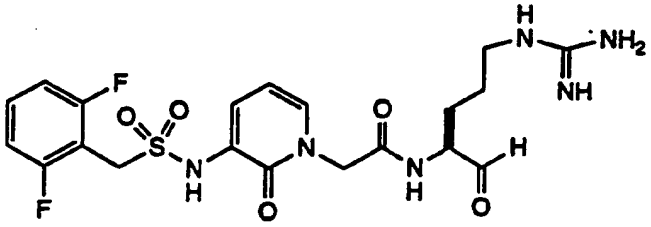
3-(3-trifluoromethylbenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121L);



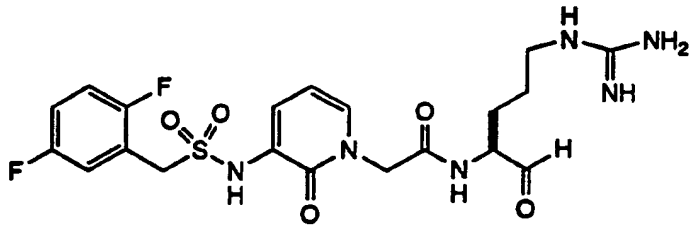
3-(2-carbomethoxybenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121M);



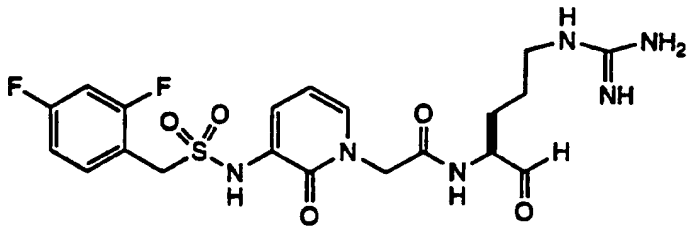
3-(3-carbomethoxybenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121N);



[3-(2,6-difluorobenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121O);



[3-(2,5-difluorobenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121P); and

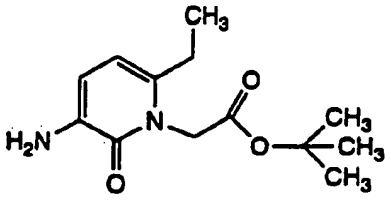


[3-(2,4-difluorobenzyl)sulfonylamino-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 121Q).

Correct molecular weight for the compounds is confirmed by mass spectroscopy.

Example 122

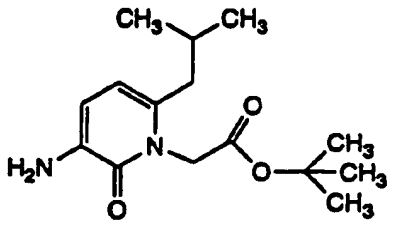
Preparation of t-Butyl (3-amino-6-ethyl-2-oxo-1,2-dihydro-1-pyridyl)acetate



Using similar procedures to that described in Example 106 herein, the title compound (5.79 g) was prepared from the compound of Example 100 (8.12 g) in quantitative yield. R_f = 0.05 (silica gel, 33% ethyl acetate/hexanes).

Example 123

Preparation of t-Butyl (3-amino-6-ethyl-2-oxo-1,2-dihydro-1-pyridyl)acetate

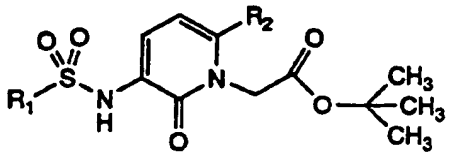


Following the four step protocol as outlined in Examples 97 to 100, substituting 2-bromopropane (10.3 mL, 0.10 mol) for iodomethane, the title compound is prepared. R_f=0.69, (silica gel, 50% ethyl acetate/hexanes).

Example 124

General Procedure for Reaction of t-Butyl (3-amino-6-alkyl-2-oxo-1,2-dihydro-1-pyridyl)acetate with sulfonyl chlorides

To a stirred solution of the compound of Example 94 (7.15 g, 30 mmole), the compound of Example 122 (7.60 g, 30 mmole), OR the compound of Example 123 (8.50 g, 30 mmole), in dry tetrahydrofuran (300 mL) is added 2,4,6-collidine (7.93 mL, 60 mmole) and the solution is cooled to 0°C under nitrogen. The appropriate sulfonyl chloride, listed below, (33 mmole) dissolved in tetrahydrofuran (25 to 75 mL) is added dropwise. After the addition is complete, the reaction mixture is stirred for 30 minutes to 1 hour at 0°C and then at ambient temperature for 0 to 72 hours. The reaction mixture is diluted with ethyl acetate, washed successively with 1.0N HCl, water, saturated sodium bicarbonate and brine, dried over magnesium sulfate, and the solvent is removed *in vacuo*. The residue is chromatographed on silica gel using a gradient system of dichloromethane and 1 to 4% methanol in dichloromethane to afford the product, judged pure by TLC (silica gel). Using this method and the starting materials listed below, intermediates having the formula given below are made:

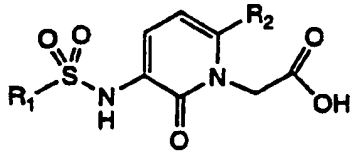


Product Compound	Starting materials
R_1=2-trifluoromethylbenzyl, R_2=CH_3	Compound of Example 94, (2-trifluoromethyl-phenyl)methanesulfonyl chloride
R_1=2-methyl-5-fluorophenyl, R_2=CH_3	Compound of Example 94, 2-methyl-5-fluorobenzene-sulfonyl chloride
R_1=2,5-dimethoxyphenyl,, R_2=CH_3	Compound of Example 94, 2,5-dimethoxybenzene-sulfonyl chloride
R_1=2-carbomethoxybenzyl, R_2=CH_3	Compound of Example 94, 2,6-difluorobenzenesulfonyl chloride
R_1=benzyl, R_2=CH_2CH_3	Compound of Example 122, benzylsulfonyl chloride
R_1=2-methyl-5-fluorophenyl, R_2=CH_2CH_3	Compound of Example 122, 2-methyl-5-fluorobenzene-sulfonyl chloride
R_1=benzyl, R_2=$CH_2CH(CH_3)_2$	Compound of Example 123, benzylsulfonyl chloride

Example 125

General Procedure for Reaction of (3-amino-6-alkyl-2-oxo-1,2-dihydropyridyl)acetic acid with trifluoroacetic acid

The intermediates of Example 124 (3.0 mmole) are treated with 50% trifluoroacetic acid/dichloromethane (10 mL). After 1 hour, the reaction mixture is concentrated, then diluted with toluene. The solution is again concentrated, toluene is added, and the solvent is removed *in vacuo*. Using this method and the starting materials listed below, intermediates having the formula given below are made:

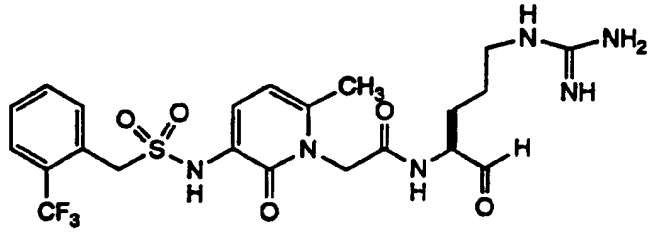


R_1=2-trifluoromethylbenzyl, R_2=CH_3;
R_1=2-methyl-5-fluorophenyl, R_2=CH_3;
R_1=2,5-dimethoxyphenyl, R_2=CH_3;
R_1=2-carbomethoxybenzyl, R_2=CH_3;
R_1=benzyl, R_2=CH_2CH_3;
R_1=2-methyl-5-fluorophenyl, R_2=CH_2CH_3; and
R_1=benzyl, R_2=$CH_2CH(CH_3)_2$.

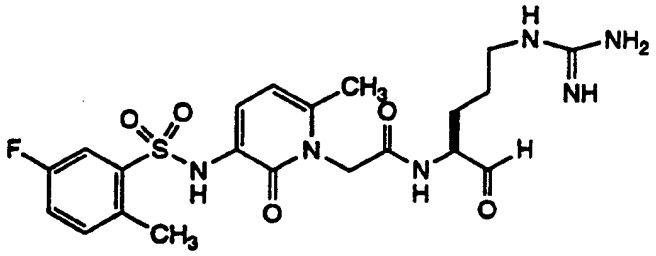
Example 126

General Procedure for the Preparation of Compounds of the Present Invention:

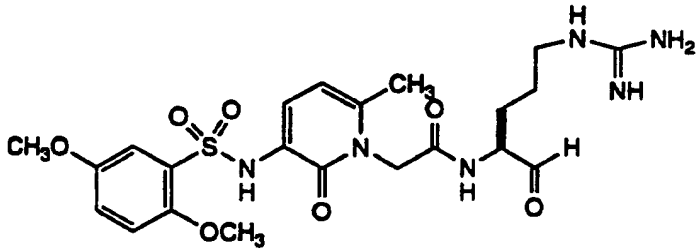
Following the three-step protocol outlined in Examples 8 to 10 (coupling, hydrogenation and hydrolysis), the intermediates of Example 125 were used to synthesize the following compounds of the present invention (as their trifluoroacetic acid salts):



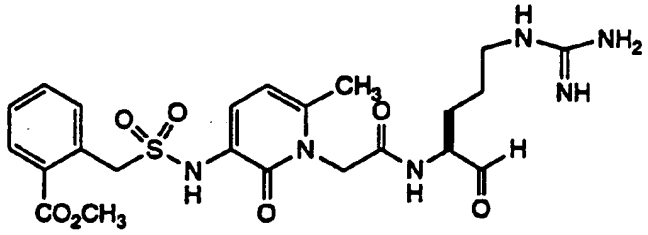
[3-(2-trifluoromethylbenzyl)sulfonylamino-6-methyl-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 126A);



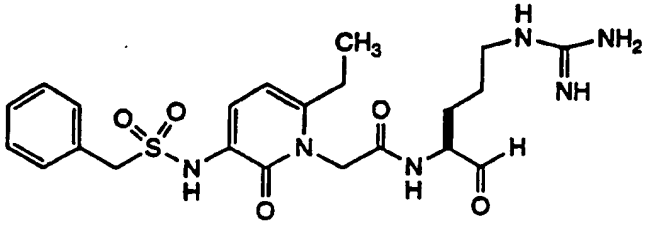
[3-(5-fluoro-2-methylphenyl)sulfonylamino-6-methyl-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 126B);



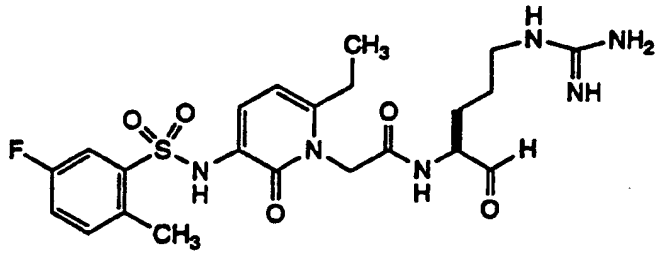
3-[(2,5-dimethoxyphenyl)sulfonylamino-6-methyl-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 126C);



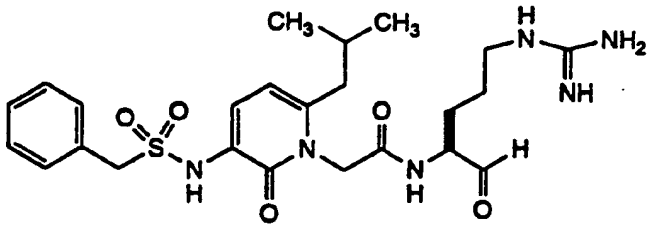
[3-(2-carbomethoxybenzyl)sulfonylamino-6-methyl-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 126D);



(3-benzylsulfonylamino-6-ethyl-2- xo-1,2-dihydro-pyridyl)acetyl-L-argininal (Compound 126E);



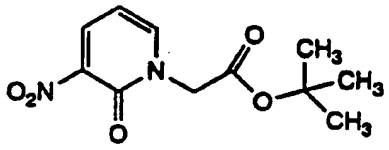
[3-(5-fluoro-2-methylphenyl)sulfonylamino-6-ethyl-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 126F); and



(3-benzylsulfonylamino-6-isobutyl-2-oxo-1,2-dihydro-pyridyl)acetyl-L-argininal (Compound 126G).

Example 127

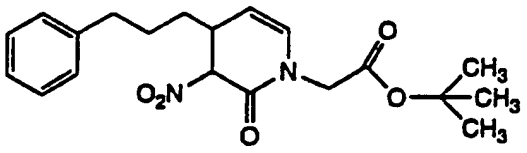
Preparation of t-butyl [3-nitro-2-oxo-1,2-dihydropyridyl]acetate



Using similar procedures to those described in Example 5 herein, the title compound (6.77 g) was prepared from 3-nitro-2-hydroxypyridine (5.00 g) in 74% yield. R_f = 0.60 (silica gel, 20% ethyl acetate/dichloromethane).

Example 128

Preparation of t-Butyl [3-nitro-2-oxo-4-(3-phenylpropyl)-1,2,3,4-tetrahydropyridyl]acetate

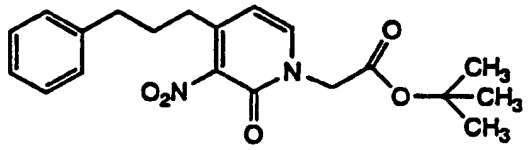


To a suspension of magnesium turnings (3.00 g, 49.6 mmole) in tetrahydrofuran (30 mL) was added (3-bromopropyl)benzene (9.87 g, 49.6 mmole) in tetrahydrofuran (20 mL) under a nitrogen atmosphere. After approximately 2 to 3 ml of the (3-bromopropyl)benzene solution were added, a few milligrams of iodine were added, and the reaction mixture was heated gently for 3 minutes. The rest of the (3-bromopropyl)benzene solution was added over 10 minutes. The solution was stirred in a 50 to 60°C oil bath overnight to give a solution of 3-phenylpropyl magesium bromide.

A solution of the compound of Example 127 (6.00 g, 23.6 mmol) and zinc chloride (6.44g, 47.2 mmole) in tetrahydrofuran (50 mL) under an argon atmosphere was stirred for 10 minutes, then was cooled in an ice bath. The solution of 3-phenylpropyl magesium bromide from this Example was added over a 5 minute period. After stirring 5 minutes at 0°C, the ice bath was removed, and the solution was allowed to stir overnight. The reaction mixture was poured into ethyl acetate (300 mL) and saturated citric acid (50 mL). The organic layer was washed with saturated sodium bicarbonate, water, and then brine (30 mL each). The solution was dried over sodium sulfate, the solvent was removed, and the residue was chromatographed through silica gel using 0 to 60% ethyl acetate/hexanes to afford 6.0 g (68% yield) of the title compound. R_f = 0.71 (silica gel, 50% ethyl acetate/hexanes).

Example 129

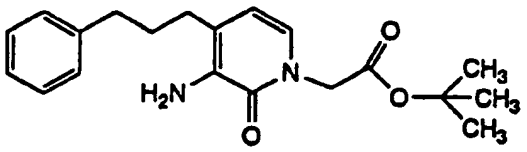
Preparation of t-Butyl [3-nitro-2-oxo-4-(3-phenylpropyl)-1,2-dihydropyridyl]acetate



The compound of Example 128 (6.0 g, 16 mmole) was dissolved in tetrahydrofuran (50 mL) under a nitrogen atmosphere. Cesium carbonate (6.3 g, 19.2 mmole, 1.2 equivalents) was added, and the solution was stirred for 10 minutes. Palladium acetate (3.63 g, 16.2 mmole, 1 equivalent) was added, and the solution was stirred overnight. Celite and silica gel were added, and the reaction mixture was filtered to remove the cesium and palladium salts, washing the solid with ethyl acetate (500 mL). The solvent was removed from the filtrate, and the residue was chromatographed on silca gel using 0 to 50% ethyl acetate/hexanes as eluent to afford 2.86 g of the title compound in 48% yield. R_f = 0.52 (silica gel, 50% ethyl acetate/hexanes).

Example 130

Preparation of t-Butyl [3-amino-2-oxo-4-(3-phenylpropyl)-1,2-dihydropyridyl]acetate

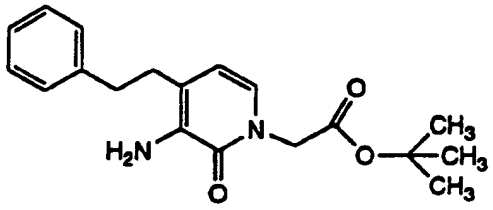


The compound of Example 129 (1.0 g, 2.7 mmole) in methanol (20 mL) was hydrogenated under balloon pressure over 10% palladium on carbon (0.15 g) for 2 hours. The reaction mixture was filtered, the solvent was removed *in*

vacuo to give the title compound (0.92 g) in quantitative yield. R_f = 0.42 (silica gel, 50% ethyl acetate/hexanes).

Example 131

Preparation of t-butyl [3-amino-2-oxo-4-(2-phenylethyl)-1,2-dihydropyridyl]acetate



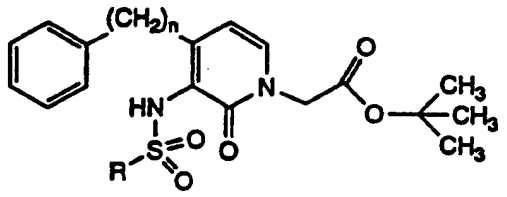
Following the three step protocol, substituting (2-bromoethyl)benzene (6.77 mL, 49.6 mmole) for (3-bromopropyl)benzene, as outlined in Examples 128 to 130, the title compound is prepared. R_f=0.63, (silica gel, 10% isopropanol/dichloromethane).

Example 132

General Procedure for Reaction of t-butyl (3-amino-4-alkyl-2-oxo-1,2-dihydropyridyl)acetate with sulfonyl chlorides.

To a stirred solution of the compound of Example 130 (10.2 g, 30 mmole) OR the compound of Example 131 (9.78 g, 30 mmole), in dry tetrahydrofuran (300 mL) is added the 2,4,6-collidine (7.93 mL, 60 mmole) and the solution is cooled to 0°C under nitrogen. The appropriate sulfonyl or sulfamoyl chloride listed below (33 to 150 mmole) dissolved in tetrahydrofuran (25 to 75 mL) is added dropwise. After the addition is complete, the reaction mixture is stirred for 30 minutes to 1 hour at 0°C and then at ambient temperature for 0 to 72 hours. The reaction mixture is diluted with ethyl acetate, washed successively with 1.0N HCl, wat r, saturated sodium bicarbonate and brine, dried over magnesium sulfate, and

the solvent is removed *in vacuo*. The residue is chromatographed on silica gel using a gradient system of dichloromethane and 1 to 4% methanol in dichloromethane to afford the product, judged pure by TLC (silica gel). Using this method and the starting materials listed below, intermediates having the formula given below are made:



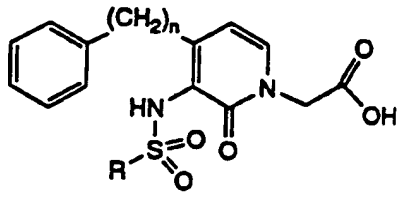
Intermediate	Starting materials
n=3, R=methyl	Compound of Example 130, methanesulfonyl chloride
n=2, R=methyl	Compound of Example 131, methanesulfonyl chloride
n=2, R=2,2,2-trifluoro-ethyl	Compound of Example 131, 2,2,2-trifluoroethanesulfonyl chloride
n=2, R=phenyl	Compound of Example 131, benzenesulfonyl chloride
n=2, R=methylamino	Compound of Example 131, methylsulfamoyl chloride

Example 133

General Procedure for Reaction of (3-amino-6-alkyl-2-oxo-1,2-dihydropyridyl)acetic acid with trifluoroacetic acid

The intermediates of Example 132 (3.0 mmole) are treated with 50% trifluoroacetic acid/dichloromethane (10 mL). After 1 hour, the reaction mixture is concentrated, then diluted with toluene. The solution is concentrated,

toluene is added, and the solvent is removed in vacuo. Using this method and the starting materials listed below, intermediates having the formula given below are made:

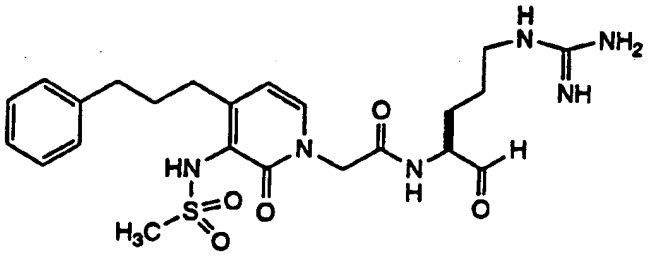


n=3, R=methyl;
n=2, R=methyl;
n=2, R=2,2,2-trifluoroethyl;
n=2, R=phenyl; and
n=2, R=methylamino.

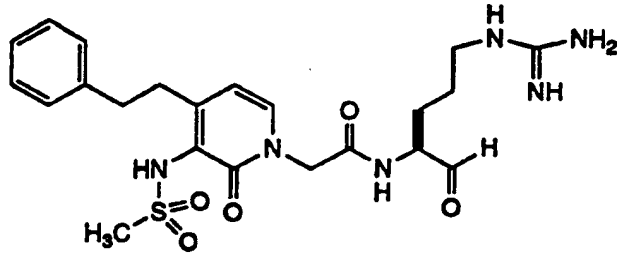
Example 134

General Procedure for the Preparation of Compounds of the Present Invention

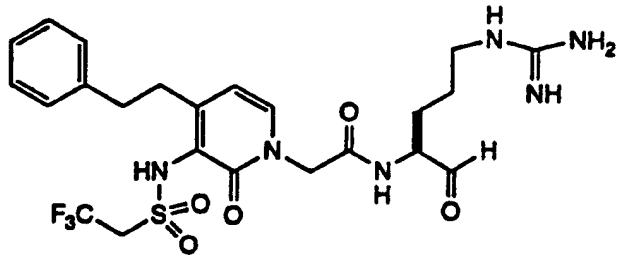
Following the three-step protocol outlined in Examples 8 to 10 (coupling, hydrogenation and hydrolysis), the intermediates of Example 133 were used to synthesize the following compounds of the present invention (as their trifluoroacetic acid salts):



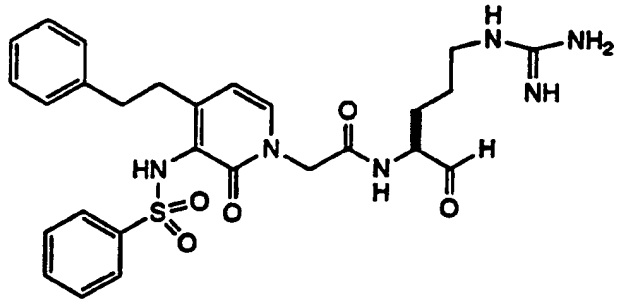
[3-methylsulfonylamino-4-(3-phenylpropyl)-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 134A);



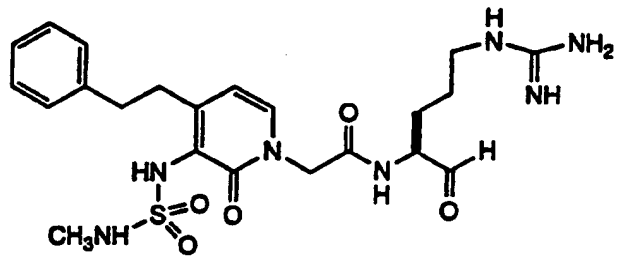
[3-methylsulfonylamino-4-(2-phenylethyl)-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 134B);



[3-(2,2,2-trifluoroethyl)sulfonylamino-4-(2-phenylethyl)-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 134C);



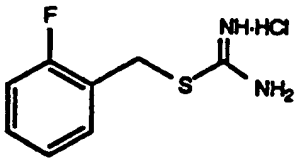
[3-phenylsulfonylamino-4-(2-phenylethyl)-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 134D); and



[3-methylaminosulfonylamino-4-(2-phenylethyl)-2-oxo-1,2-dihydro-pyridyl]acetyl-L-argininal (Compound 134E).

Example 135

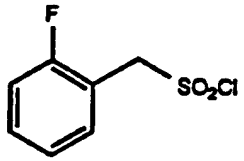
Preparation of 2-Fluorobenzyl thiouronium hydrochloride



2-Fluorobenzyl chloride (125 g, 0.86 mole, Aldrich), thiourea (66 g, 0.87 mole) and 200 mL of methanol were refluxed for 2 hours under nitrogen. The reaction was cooled and the volume was reduced to ~40 mL. The slurry was poured into 1 liter of diethyl ether. The resulting white solid was filtered and dried under vacuum to give 184.5 g (97 %) of the title compound as a white solid.

Example 136

Preparation of (2-fluorophenyl)methanesulfonyl chloride

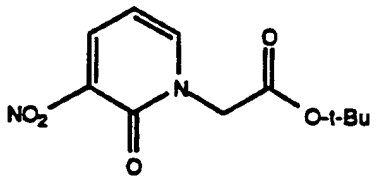


The compound of Example 135 (184.5 g, 0.836 mole) was dissolved in 1700 mL of distilled water. The reaction was cooled to -5°C in an dry ice/acetone bath. Chlorine gas was bubbled through the solution while stirring with a mechanical stirrer; the reaction temperature was maintained at -5°C to 5°C throughout the addition of chlorine gas. A sodium bisulfite/water trap removed the excess chlorine gas. The chlorine gas was added until the point of saturation was reached, there was no rise in temperature and the reaction pale green color was maintained. The resulting solids were filtered and the solids were dissolved in 1 lit r of ether. The ether layer was washed with dilute sodium bisulfite ($NaHSO_3$) four

times to remove the excess chlorine. The ether layer was dried over magnesium sulfate, filtered and concentrated under vacuum to yield 156 g (89.4 %) of the title compound as a white solid.

Example 137

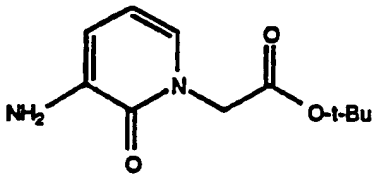
Preparation of t-Butyl (3-nitro-2-oxo-1,2-dihydropyridyl)acetate



2-Hydroxy-3-nitro-pyridine (75 g, 0.535 mole) and 1400 mL of anhydrous tetrahydrofuran were stirred at 0°C using a mechanical stirrer. Lithium bis(trimethylsilyl)amide (1.0 M solution in tetrahydrofuran, 683.5 mL) was slowly added over 30 minutes. The deep brown reaction mixture was stirred for 30 additional minutes and then t-butyl bromoacetate (109.6 g, 0.561 mole) was slowly added over 30 minutes. The reaction mixture was warmed to 25°C overnight. The organic solvents were removed under vacuum and the residue was dissolved in 2 liters of ethyl acetate and 500 mL of water. The organic phase was dried with magnesium sulfate, filtered and evaporated under vacuum. The residue was chromatographed on silica gel using a methylene chloride:ethanol gradient, 100:0 to 98:2, to yield 102.1 g (75 %) of the title compound as a yellow-orange solid.

Example 138

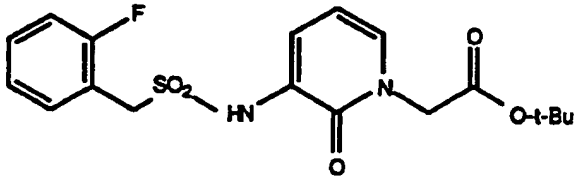
Preparation of t-Butyl(3-amino-2-oxo-1,2-dihydropyridyl)acetate



The compound of Example 137 (54 g, 0.213 mole) and 880 ml of methanol and 5 g of 10% palladium on carbon were stirred under 1 Atm of hydrogen for 24 hours. The reaction mixture was filtered and the carbon was washed with 200 mL of dichloromethane. The organic layer was evaporated to yield 47.08 g (98.5 %) of the title compound as a brown solid.

Example 139

Preparation of t-Butyl[3-(2-fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydropyridyl]-acetate

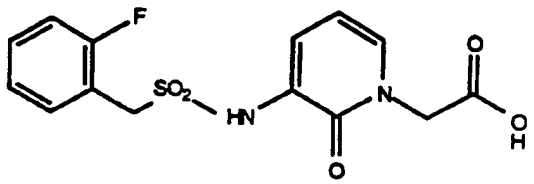


A mixture of the compound of Example 136 (22.27 g, 0.107 mole), the compound of Example 138 (23.94 g, 0.107 mole), and 150 mL of acetonitrile was cooled to 0°C and 4-methylmorpholine (NMM) (58.68 mL, 0.53 mole) was slowly added over 15 minutes. The reaction mixture was warmed to 25°C overnight. The solvent was evaporated under vacuum and the residue was dissolved in 400 mL of ethyl acetate and 100 mL of water. The organic layer was separated and washed 3 times with 100 mL of 1 N HCl, $NaHCO_3$ (saturated), and brine. The organic layer was dried over magnesium sulfate, filtered and evaporated under reduced pressure. Th dark brown residue showed 3 spots by TLC. Th residue was crystallized from dichloromethane. Two crops of white solid were obtained, 13.70 and 8.39 g,

respectively. The mother liqu rs were evaporated and the resulting dark brown solid was crystallized from dichloromethane to yield 5.28 g of a white solid. The three crops were combined and yielded 27.37 g (64.7%) of the title compound.

Example 140

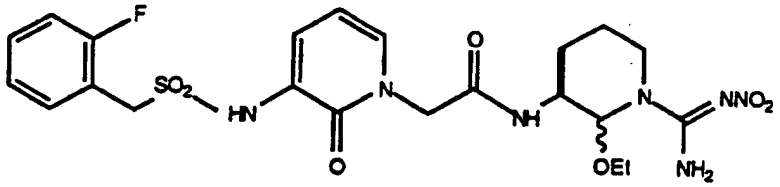
Preparation of 3-(2-Fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydropyridyl)acetic acid



The compound of Example 139 (13.97 g, 0.35 mole) and 50 mL of dichloromethane were cooled to 0°C. Trifluoroacetic acid (TFA)(50 mL) was added and the reaction was stirred for 2 hours. The reaction was judged complete by TLC and the solvent was removed under vacuum. To remove all traces of TFA, 100 mL of toluene and 500 mL of dichloromethane were added and the solvents were removed under vacuum. The residue was dried under vacuum overnight to yield 11.9 g (99 %) of the title compound.

Example 141

Preparation of [3-(2-Fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-N^{g}-nitro-L-argininal ethyl cyclol

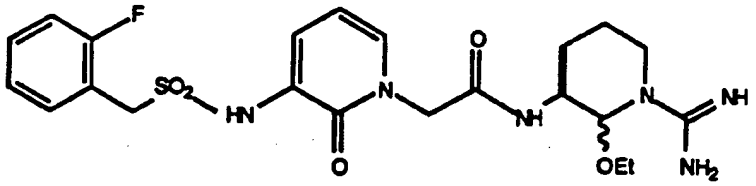


The compound of Example 140 (22.0 g, 0.65 mole), EDC (14.87 g, 0.078 mole), HOBT (10.48 g, 0.078 mole) and 250 mL of acetonitrile were stirred for 15 minutes at 25°C.

This mixture was cooled to 0°C and the compound f Example 4, N^g-nitro-L-argininal ethyl cyclol (17.31 g, 0.065 mol) was added. To this suspension was added slowly NMM (35.5 mL, 0.323 mol). After the addition of the NMM, the reaction mixture became a golden brown. The reaction mixture was allowed to warm to 25°C overnight. The solvent was removed under vacuum; the residue was dissolved in 500 mL of dichloromethane and 100 mL of water. The organic layer was separated and washed 3 times with 100 mL of 1 N HCl, $NaHCO_3$ (saturated) and 100 mL of brine. The organic phase was dried over magnesium sulfate, filtered and evaporated under reduced pressure to give 26 g of a brown foam. The residue was chromatographed on silica gel eluting with dichloromethane:methanol gradient 100:0 to 97:3, to yield 19.69 g (55%) of the title compound.

Example 142

Preparation of [3-(2-Fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ethyl cyclol, acetate salt

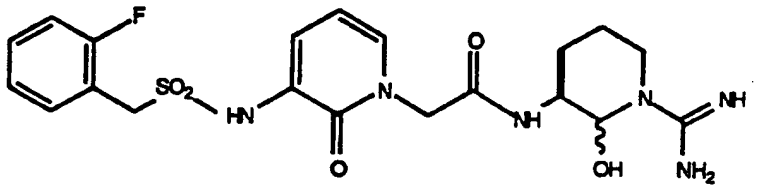


The compound of Example 141 (13.3 g, 0.024 mole), 80 ml of ethanol:acetic acid (4:1), and 2 g of 10% palladium on carbon were stirred overnight under 1 Atm of hydrogen. The carbon was filtered, washed with 100 mL of dichloromethane and the organic solvents were removed under vacuum. The resulting brown oil was dissolved in 100 mL of dichloromethane and the solvent was removed under vacuum. The resulting glass was dried under vacuum overnight, to yield 13.6 g (>100 %, theoretical yield 12.3

g) of the title compound. The product contained acetic acid trapped in the glass.

Example 143

Preparation of [3-(2-fluorobenzylsulfonyl)amino-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal



The compound of Example 142 (13.6 g, 12.3 g theoretical yield, 0.024 mole) was cooled to 0°C and 123 mL of 8 N HCl at 0°C was added. The reaction mixture was stirred for 40 minutes and checked by HPLC for completion of the reaction. After the reaction was judged complete by HPLC, 133.9 g of sodium acetate in 150 mL of water was added to give a pH of ~4. The solution was filtered through a 0.2 micron nylon filter and chromatographed on a 4" Vydac C18 column using the following solvent gradient of CH_3CN(B) - 99.9% H_2O/0.1%TFA(A) for elution: 0% to 17% B over 10 minutes and 17% to 23% B over 30 minutes. Two major fractions of the title compound were obtained: 3.86 g (33.2%) > 95% pure and 4.75 g (40.8%) ~90 to 95% pure.

Example A

Kinetic analysis of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal in an *in vitro* thrombin inhibition assay

The ability of a compound of a present invention, [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal (Example 10), to act as an inhibitor of thrombin catalytic activity was assessed by determining its inhibition constant, Ki.

Enzyme activity was determined using the chromogenic substrate Pefachrome t-PA (CH_3SO_2-D-hexahydrotyrosine-

glycyl-L-Arginine-p-nitroaniline), obtained from Pentapharm Ltd. The substrate was reconstituted in deionized water prior to use. Purified human alpha-thrombin (3000U/mg specific activity) was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for Ki determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration diluted in HBSA (or HBSA alone for Vo(uninhibited velocity) measurement), and 50 microliters of the chromogenic substrate (250 micromolar, 5-times Km). At time zero, 50 microliters of alpha-thrombin diluted in HBSA were added to the wells, yielding a final concentration of 0.5 nM in a total volume of 200 microliters. Velocities of chromogenic substrate hydrolysis which occurred over 40 minutes were measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader. Ki values were determined for test compounds using the relationships developed by Williams and Morrison, Methods in Enzymology, 63:437 (1979) using steady state velocities (Vs) measured over 40 minutes. The extent of substrate hydrolysis was less than 5% over the course of this assay.

Table 1 below gives the Ki values for [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal. The data show the utility of this compound as a potent *in vitro* inhibitor of human alpha-thrombin.

Table 1. Inhibitor constant of [3-[(benzylsulfonyl) amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal against human alpha-thrombin amidolytic activity

Compound	Ki (pM)
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal	289±32

Example B

In vitro enzyme Assays for specificity determination

The ability of compounds of the present invention to act as a selective inhibitor of thrombin catalytic activity was assessed by determining the concentration of compound which inhibited the activity of this enzyme by 50%, (IC_{50}), and comparing this value to that determined for all or some of the following related serine proteases: recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor Xa and trypsin.

The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for IC_{50} determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad concentration range) diluted in HBSA (or HBSA alone for V_o (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below were added to the wells, yielding a final total volume of 200 microliters. The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

Thrombin (fIIa) Assay

Enzyme activity was determined using the chromogenic substrate, Pefachrome t-PA (CH_3SO_2-D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroanilin , obtained from Pentapharm Ltd.). The substrate was reconstituted in deionized water prior to use. Purified human a-thrombin was obtained from

Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

IC_{50} determinations were conducted where HBSA (50 mL), a-thrombin (50 µl) and inhibitor (50 µl) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of substrate Pefachrome-t-PA (50 µl). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

Factor Xa

Factor Xa catalytic activity was determined using the chromogenic substrate S-2765 (N-benzyloxycarbonyl-D-arginine-L-glycine-L-arginine-p-nitroaniline), obtained from Kabi Pharmacia Hepar, Inc. (Franklin, OH). All substrates were reconstituted in deionized water prior to use. The final concentration of S-2765 was 250 µM (about 5-times Km). Purified human Factor X was obtained from Enzyme Research Laboratories, Inc. (South Bend, IN) and Factor Xa (FXa) was activated and prepared from it as described [Bock, P.E., Craig, P.A., Olson, S.T., and Singh, P. Arch. Biochem. Biophys. 273:375-388 (1989)].

Recombinant tissue plasminogen activator (rt-PA) Assay

rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA (CH_3SO_2-D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to th assay in which the final concentration was 500 micromolar (about 3-times Km). Human rt-PA (Activase®) was obtained from Genentech Inc. The enzyme was reconstituted in deionized water and

diluted int HBSA prior to the assay in which the final concentration was 1.0 nM.

Plasmin Assay

Plasmin catalytic activity was determined using the chromogenic substrate, S-2251 [D-valyl-L-leucyl-L-lysine-p- nirtoanilide dihydrochloride], which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times Km). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Activated Protein C (aPC) Assay

aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC (delta-carbobenzloxy-D-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride), obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 3-times Km). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Chymotrypsin Assay

Chymotrypsin catalytic activity was determined using the chromogenic substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times Km). Purified (3X-crystallized;CDI) bovine pancreatic alpha-chymotrypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA

pri r to assay in which the final concentration was 1.0 nM.

Trypsin Assay

Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid-[gamma-methyl ester]-L-arginine-p-nitroanilide), which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times Km). Purified (3X-crystallized; TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

Tables 2, 3A and 3B list the determined IC_{50} values for certain of the enzymes listed above and demonstrate the high degree of specificity for the inhibition of alpha-thrombin compared to these related serine proteases.

Table 2 IC_{50} values (nM) for the inhibition of human alpha thrombin amidolytic activity compared to selected serine proteases for compounds of Example 10 (column A), Example 90, compound B (column B), and Example 113, compounds C, D, and E (columns C, D, and E, respectively)

Enzyme	A	B	C	D	E
Alpha-thrombin	0.66	0.98	0.467	2.32	141
rt-PA	NI*	NI*	ND	NI*	NI*
Plasmin	NI*	NI*	NI*	NI*	NI*
aPC	NI*	NI*	ND	NI*	NI*

NI*- IC_{50} value >2500 nM.

ND - not determined

Tables 3A and 3B — IC_{50} values (nM) for inhibition of human alpha thrombin amidolytic activity compared to inhibition of rt-PA, plasmin, and aPC for compounds made according to Examples 89 and 90 with the stated R_1 substitution

Table 3A

R_1 substitution	Thrombin (IC_{50})	rt-PA (IC_{50})	Plasmin (IC_{50})	aPC (IC_{50})
2-CF_3-phenyl	5.6	NI*	NI*	NI*
3-CF_3-phenyl	3.1	NI*	NI*	NI*
2-Me-phenyl	1.4	NI*	NI*	NI*
3-Me-phenyl	0.85	NI*	NI*	NI*
2-Me,5-F-phenyl	1.97	NI*	NI*	NI*
2-OMe-phenyl	1.98	NI*	NI*	NI*
3-OMe-phenyl	0.65	NI*	NI*	NI*
2-OMe,5-Cl-phenyl	1.16	NI*	NI*	NI*
2-NH_2-phenyl	3.7	NI*	NI*	NI*

Table 3B

Compound of Example	Thrombin	rtPA	Plasmin	aPC
121A	14.7	-	>2500	-
121E	.597	inactive	>2500	inactive
121G	46.9	-	~2500	-
121J and 143	.763	>2500	>2500	inactive
121K	.882	inactive	>2500	inactive
121M	.623	-	>2500	-
121P	1.73	>2500	>2500	inactive
126B	.882	inactive	>2500	inactive
126C	.519	inactive	>2500	inactive
126F	.71	inactive	~2500	inactive
134B	7.95	>2500	>2500	inactive

The demonstrated anticoagulant effects f a compound of the present invention, [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, in human citrated plasma indicated that this compound may have potent antithrombotic effects in an experimental model of thrombosis. To investigate this, the antithrombotic (prevention of thrombus formation) properties of this compound were evaluated using the following established experimental model of acute vascular thrombosis.

Rat model of $FeCl_3$-induced platelet-dependent arterial thrombosis

This is a well characterized model of platelet dependent, arterial thrombosis which has been used to evaluate the potential of antithrombotic compounds such as direct thrombin inhibitors. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60:269-280 (1990). In this model a platelet-rich, occlusive thrombus is formed in a segment of the rat carotid artery treated locally with a fresh solution of $FeCl_3$ absorbed to a piece of filter paper. The $FeCl_3$ is thought to diffuse into the treated segment of artery and cause de-endothelialization of the affected vessel surface. This results in the exposure of blood to subendothelial structures which in turn causes platelet adherence, thrombin formation and platelet aggregation resulting in occlusive thrombus formation. The effect of a test compound on the incidence of occlusive thrombus formation following the application of the $FeCl_3$ is monitored by ultrasonic flowtometry and is used as the primary end point. The use of flowtometry to measure carotid artery blood flow, is a modification of the original procedure in which thermal detection of clot formation was employed. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60:269-280 (1990).

Male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use and fasted for 12 hours prior to surgery with free access to water. The animals were prepared, anesthetized with Nembutal followed

experimental model of acute vascular thrombosis.

Rat model of $FeCl_3$-induced platelet-dependent arterial thrombosis

This is a well characterized model of platelet dependent, arterial thrombosis which has been used to evaluate the potential of antithrombotic compounds such as direct thrombin inhibitors. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60:269-280 (1990). In this model a platelet-rich, occlusive thrombus is formed in a segment of the rat carotid artery treated locally with a fresh solution of $FeCl_3$ absorbed to a piece of filter paper. The $FeCl_3$ is thought to diffuse into the treated segment of artery and cause de-endothelialization of the affected vessel surface. This results in the exposure of blood to subendothelial structures which in turn causes platelet adherence, thrombin formation and platelet aggregation resulting in occlusive thrombus formation. The effect of a test compound on the incidence of occlusive thrombus formation following the application of the $FeCl_3$ is monitored by ultrasonic flowtometry and is used as the primary end point. The use of flowtometry to measure carotid artery blood flow, is a modification of the original procedure in which thermal detection of clot formation was employed. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60:269-280 (1990).

Male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use and fasted for 12 hours prior to surgery with free access to water. The animals were prepared, anesthetized with Nembutal followed by the insertion of catheters for blood pressure monitoring, drug and anesthesia delivery. The left carotid artery was isolated by making a midline cervical incision followed by blunt dissection and spreading techniques to separate a 2 cm segment of the vessel from the carotid sheath. A silk suture is inserted under the proximal and distal ends of the isolated vessel to provide clearance for the placement of a ultrasonic flow probe

(Transonic) around the proximal end of the vessel. The probe is then secured with a stationary arm.

Following surgery the animals were randomized in either a control (saline) or treatment group with test compound, [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, with at least 6 animals per group per dose. The test compound was administered as a single intravenous bolus at the doses outlined in Table 3 after placement of the flow probe and 5 minutes prior to the thrombogenic stimulus. At t=0, a 3mm diameter piece of filter paper (Whatman #3) soaked with 10 microliters of a 35% solution of fresh $FeCl_3$ (made up in water) was applied the segment of isolated carotid artery distal to the flow probe. Blood pressure, blood flow, heart rate, and respiration were monitored for 60 minutes.

The incidence of occlusion (defined as the attainment of zero blood flow) was recorded as the primary end point.

The efficacy of the [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal as an antithrombotic agent in preventing thrombus formation in this *in vivo* model was demonstrated by the reduction in the incidence of thrombotic occlusion as shown in Table 4 below.

Table 4 Results of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal in the $FeCl_3$ Model of Thrombosis in Rats.

Treatment Group	Dose (mg/kg)	n	Incidence of Occlusion
Saline	--------	6	6/6
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl -L-argininal	0.3	6	6/6

[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal	1.0	6	3/6
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal	3.0	6	1/6*
[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal	5.0	6	0/6*

*-p≤0.05 from saline control by Fishers test

The effective dose which prevents 50% of thrombotic occlusions in this model (ED_{50}) can be determined from the above data by plotting the incidence of occlusion versus the dose administered. This allows a direct comparison of the antithrombotic efficacy of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, with other antithrombotic agents which have also been evaluated in this model as described above. Table 5 lists the ED_{50} values for several well known anticoagulant agents in this model compared to [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal.

Table 5 Efficacy of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal compared to other antithrombotic agents based on ED_{50} for thrombus prevention in the $FeCl_3$ model of arterial

Compound	ED_{50}[a]
Standard Heparin	200U/kg
Argatroban	3.8mg/kg
Hirulog™	3.0mg/kg

[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal	1.0mg/kg

[a]ED_{50} is defined as the dose that prevents the incidence of complete thrombotic occlusion in 50% of animals tested.

The data presented in Table 4 clearly demonstrate the effectiveness of a compound of the present invention, [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal, in preventing occlusive thrombus formation in this experimental model. The relevance of these data to preventing human thrombosis can be inferred from the comparison to the other anticoagulant agents listed in Table 5 which have been evaluated in an identical manner in this experimental model and have demonstrated antithrombotic efficacy in preventing thrombus formation clinically as described in the following literature citations: Heparin-Hirsh, J., N. Engl. J. Med., 324:1565-1574 (1992) and Cairns, J.A. et al., Chest, 102:456S-481S (1992); Argatroban-Gold, H.K. et al., J. Am. Coll. Cardiol., 21:1039-1047 (1993); and Hirulog™-Sharma, G.V.R.K. et al., Am. J. Cardiol., 72: 1357-1360 (1993) and Lidón, R.M. et al., Circulation, 88:1495-1501 (1993). The *in vivo* comparison of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal with the clinically effective antithrombotic agents, Standard Heparin, Argatroban, and Hirulog™, in the same rodent model of experimental thrombosis, coupled with the demonstrated anticoagulant effects of [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal in human plasma described above in Example C would lead one skilled in the art to conclude that the compounds of the present invention will be an effective antithrombotic agent in humans.

Example E

Multiple Extracorporeal Shunt Model in Rats Utilizing Oral Dosing

The compounds of Example 10 and 143 were evaluated in a multichamber A-V shunt model in rats. The A-V shunt model is one of the most common and generally used systems to evaluate antithrombotic compounds. Smith, J.R. and White, A.M. *Br. J. Pharmacol.*, 77: 29-38 (1982). In this model a localized clot made up of primarily fibrin with some platelet and macrophage involvement (Shand, R. A. and Smith, J.R. and Wallis, R. B. *Thromb. Res.*, 36: 223-232 (1984)), is formed on an artificial thrombogenic surface (typically a segment of silk or cotton thread) contained in a sialstic chamber which is part of an exteriorized shunt between the carotid artery and jugular vein. The procedure described in this Example is a modified A-V shunt model that allows for oral dosing of test agents and subsequent evaluation of efficacy over a two to three hour window in time.

Briefly, male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use. The animals were fasted for 12 hours prior to surgery with free access to water. Unanesthetized animals were grouped into three or four dosage groups (six or seven animals per group) and administered test agents orally via gavage needle, at doses of 1.0, 3.0, 10 and 50 mg/kg for the compound of Example 10, and 3.0, 10 and 30 mg/kg for the Compound of Example 143. Immediately after oral dosing, animals were anesthetized with sodium pentobarbital (Nembutal) given intraperitoneally at a dose of 50 mg/kg body weight, and placed on a isothermal pad to maintain body temperature. The level of anesthesia was monitored every 15 minutes by neuro-response to a tail pinch, respiration and core temperature. The desired depth of surgical anesthesia was maintained by administering subsequent doses (5 mg/kg) intravenously. The left femoral artery was catheterized using standard procedures for blood pressure monitoring and blood sampling, with polyethylene tubing (PE50). The left femoral vein was catheterized with PE50 tubing for delivery of anethestic.

The exteriorized shunts were assembled by connecting

two pieces of saline filled 12.5 cm PE90 tubing with a 6 cm piece of PE160 tubing containing a 6 cm piece of silk suture size 3 and clamped with hemostats. A small 0.5 cm portion of the silk thread protrudes from the junction of the chamber with the shunt. The left jugular vein and right carotid artery were catheterized with the ends of the PE90 shunt. The shunt was unclamped and blood allowed to flow from the carotid artery, through the chamber, and exit the shunt via the jugular vein. After 15 minutes, both sides of the chamber were clamped and the suture containing the clot removed following detachment of the arterial end of the chamber. The clot was immediately weighed and recorded. This procedure takes place at predetermined intervals (60, 90, 120, and 150 minutes after oral dosing) to allow assessment of efficacy over a large window in time. Four shunts were placed with flow initiated at 45, 75, 105, and 135 minutes after oral compound administration. Clot weight from the four shunts was the primary endpoint of the protocol. Blood pressure, heart rate core temperature and respiration were monitored continuously. Following termination of the experiment the animals were euthanized with a 120 mg/kg dose of Nembutal. One experiment was performed per animal.

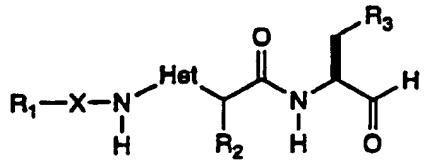
ED_{50} values were calculated at 60, 90, 120, and 150 minutes after oral dosing of test compound. ED_{50} is that dose that reduced the clot size by 50%. For the compound of Examples 10 and 143, the ED_{50} values were as shown in Table 6, below, and demonstrate the oral availability and efficacy of the compounds.

Table 6

Time after oral dose	ED_{50} value	
60 min	(Example 10) <1.0 mg/kg	(Example 143) 22 mg/kg
90 min	2.9 mg/kg	20 mg/kg
120 min	2.9 mg/kg	24 mg/kg
150 min	8.2 mg/kg	28 mg/kg

WE CLAIM:

1. A compound of formula:



wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R")-$ and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and;

(b) R_1 is selected from the group consisting of:

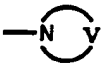
(1) alkyl of 1 to about 12 carbon atoms,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the ring carbons with hydroxyl, alkoxyl or alkyl of 1 to about 3 carbons, amino, guanidino, or amidino,

(5) heterocyclo f 4 to about 10 ring atoms with th ring atoms selected from carbon and heteroatoms, wherein th heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0,

1 or 2, and including  , wherein  is a 5 to 7 member heterocycle of 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-,optionally substituted on the ring carbons with hydroxyl, alkoxyl or alkyl each of 1 to about 3 carbons, amino, guanidino, or amidino,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms, which optionally is substituted in the ring carbons with hydroxyl, amino, guanidino, amidino, or alkoxyl or alkyl each of 1 to about 3 carbons,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

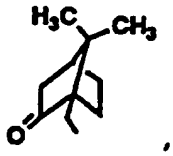
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,

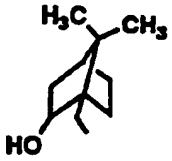
(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

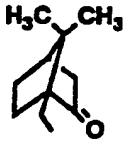
(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,

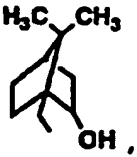
(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2,

and/or Y_3,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

(17) difluoromethyl and perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(20) hydrogen,

wherein Y_1, Y_2, and Y_3 are

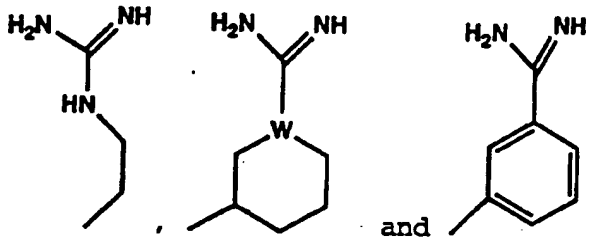
(i) independently selected from the group consisting of hydrogen, halogen, cyano, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2H$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, OCF_2CF_3, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)OZ_1$, $-P(O)_3H$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, and $-NZ_1Z_2$, wherein m is 0, 1 or 2, and Z_1 and Z_2 are

independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen,

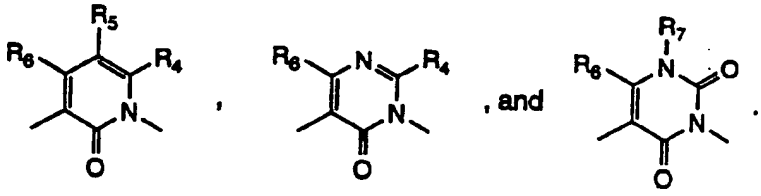
(c) R_2 is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms, and alkenyl of about 2 to about 4 carbon atoms,

(d) R_3 is selected from the group consisting of



where W is nitrogen or carbon;

(e) Het is selected from the group consisting of



wherein

(1) R_4 is selected from the group consisting of

(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, and halogen, wherein n is 0, 1 or 2, and R_1 is independently selected,

with the proviso that R_4 is not a camphor derivative or heterocyclo group,

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_5 wherein Z_5 is selected from the group consisting of hydroxy, halogen, $-C(O)OH$, $-C(O)OR_8$, $-S(O)_3OH$, and $-S(O)_pR_8$ wherein R_8 is alkyl of 1 to about 6 carbon atoms and p is 0, 1 or 2, and

(c) alkenyl of about 3 to about 6 carbon atoms;

(2) R_5 is selected from the group consisting of

(a) hydrogen,

(b) alkyl of 1 to about 10 carbon atoms,

(c) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 3 to about 8 carbon atoms,

(d) cyclic alkyl of 3 to about 6 carbon atoms,

(e) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2,

(f) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is attached to Het by a ring carbon atom,

(g) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of 3 to about 5 carbon atoms,

(h) aryl which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3 respectively,

(i) heteroaryl of 5 to 6 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $-S(O)_i-$ wherein i is

independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted with Y_1, Y_2 and/or Y_3,

(j) aralkyl of about 7 to about 10 carbon atoms which is optionally mono-, di- or tri-substituted on the aryl ring with Y_1, Y_2 and/or Y_3;

(k) heteroaralkyl of 6 to 9 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen and $-S(O)_i-$ wherein i is independently 0, 1 or 2 and which is optionally mono-, di- or tri- substituted on the ring with Y_1, Y_2 and/or Y_3,

(l) aralkenyl of 8 carbon atoms which is optionally mono-, di- or tri- substituted on the aryl ring with Y_1, Y_2 and/or Y_3,

(m) heteroaralkenyl of 7 to 8 atoms with the ring atoms selected from carbon atoms and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $-S(O)_i-$ wherein i is independently 0, 1 or 2, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3,

(n) halogen,

(o) difluoromethyl or perfluoroalkyl of 1 to 3 carbon atoms,

(p) perfluorophenyl,

(q) perfluoroaralkyl of 7 to about 9 carbon atoms, and

(r) alkoxy of 1 to about 10 carbon atoms;

(3) R_6 is selected from the group consisting of

(a) R_1, $-OR_1$, $-NHR_1$, $-S(O)_nR_1$, or halogen, wherein n is 0, 1 or 2, and R_1 is independently selected, with the proviso that R_6 is not a camphor derivative or heterocyclo group, and

(b) alkyl of 1 to about 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, $-OR_9$, $-NHR_9$, $-C(O)OH$, $-C(O)OR_9$, $-S(O)_2OH$ and $-S(O)_pR_9$ wherein R_9 is selected from alkyl of 1 to about 12 carbon atoms, aryl of

about 6 to about 10 carbon atoms opti nally mono-, di or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 12 carbon atoms optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, heteroaryl of 1 to about 9 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and heteroaralkyl of about 2 to about 10 carbon atoms with the ring atoms selected from carbon and heteroatoms selected from the group consisting of oxygen, nitrogen and $-S(O)_p-$ and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3; and

(4) R_7 is independently selected from the R_5 group of substituents, provided that R7 is not halogen; and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein X is selected from the group consisting of $-SO_2-$, $-NH-S(O)_2-$, and $-N(R')-S(O)_2-$.

3. A compound according to claim 2, wherein X is $-SO_2-$.

4. A compound according to claim 1, wherein R_1 is selected from the group consisting of alkyl, cycloalkyl, aralkyl, and aryl.

5. A compound according to claim 4, wherein R_1 is selected from the group consisting of substituted or unsubstituted phenyl, benzyl, and naphthyl.

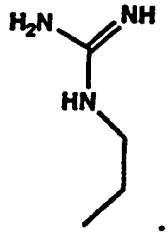
6. A compound according to claim 5, wherein R_1 has one or two substituents selected from the group consisting f methyl, methoxy, fluoro, chloro, trifluoromethyl, and $-OCF_3$.

7. A compound according to claim 4, wherein R_1 is

cycl hexyl r cyclohexylmethyl.

8. A compound according to claim 1, wherein R_2 is hydrogen.

9. A compound according to claim 1 wherein R_3 is



10. A compound according to claim 9, wherein R_4 is selected from the group consisting of:

(a) hydrogen,

(b) alkyl of 1 to 6 carbon atoms or alkyl of 1 to 6 carbon atoms substituted with Z_5, wherein Z_5 is selected from the group consisting of hydroxy, halogen, $-C(O)OH$, $-C(O)OR_8$, $-S(O)_2OH$ and $-S(O)_pR_8$,

(c) alkyl of 1 to 3 carbon atoms substituted with cyclic alkyl of 3 to 5 carbon atoms,

(d) alkenyl of about 3 to about 6 carbon atoms,

(e) cycloalkyl of about 3 to about 5 carbon atoms,

(f) heteroaryl of 5 atoms, and

(g) heteroaralkyl of 6 atoms.

11. A compound according to claim 9, wherein R_5 is selected from the group consisting of hydrogen, alkyl of 1 to about 5 carbon atoms, trifluoromethyl, and alkoxy of 1 to 4 carbon atoms.

12. A compound according to claim 11, wherein R_5 is hydrogen.

13. A compound according to claim 9, wherein R_6 is selected from the group consisting of:

(a) hydrogen,

(b) alkyl of 1 to about 12 carbon atoms or alkyl of 1 to 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, $-OR_9$, $-NHR_9$, $-C(O)OH$, $-C(O)OR_9$, $-S(O)_2OH$ and $-S(O)_pR_9$.

(c) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms,

(d) alkenyl of about 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms, or aryl of about 6 to about 10 carbon atoms,

(e) aralkyl or substituted aralkyl,

(f) heteroaralkyl of about 5 to 10 ring atoms or substituted heteroaralkyl of about 5 to 10 ring atoms,

(g) aralkenyl of about 8 to 15 carbon atoms which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, and

(h) heteroaralkenyl of about 5 to 10 ring atoms or substituted heteroaralkenyl of about 5 to 10 ring atoms.

14. A compound according to claim 13, wherein R_4 and R_5 are hydrogen or methyl and R_6 is selected from the group consisting of aralkyl of about 8 to about 13 carbon atoms, and -O-aralkyl, -NH-aralkyl, or $-S(O)_p$-aralkyl all of about 7 to about 12 carbon atoms.

15. A compound according to claim 14, wherein the aryl portion of the aralkyl group of R_6 is selected from unsubstituted or substituted phenyl or naphthyl.

16. A compound according to claim 15, wherein said substituents of the aryl ring are selected from the group consisting of methyl, methoxy, fluoro, chloro and trifluoromethyl.

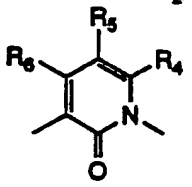
17. A compound according to claim 13, wherein R_6 is

selected from the group consisting of phenylethyl, phenylpropyl, cyclohexylethyl and cyclohexylpropyl.

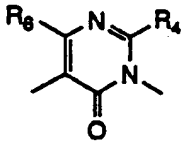
18. A compound according to claim 9, wherein R_7 is selected from the group consisting of hydrogen, methyl, difluoromethyl and trifluoromethyl.

19. A compound according to claim 18, wherein R_7 is hydrogen.

20. A compound according to claim 9, wherein Het is

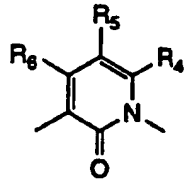


or



.

21. A compound according to claim 20, wherein Het is

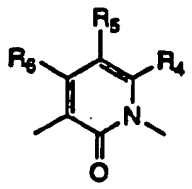


,

wherein R_4 is selected from the group consisting of hydrogen, methyl, ethyl, propenyl, allyl, propyl, isopropyl, butyl, R-sec-butyl, S-sec-butyl, isobutyl, 1-pentyl, R-2-pentyl, S-2-pentyl, 3-pentyl, S-1-(2-methyl)-butyl, R-2-(3-methyl)-butyl, 1-(3-methyl)-butyl, R-1-(2-methyl)-butyl, cyclopentyl, 2-pyrolyl, 3-pyrolyl, 1-hexyl, S-2-hexyl, R-2-hexyl, R-3-hexyl, and S-3-hexyl; and R_5 and R_6 are independently selected from hydrogen and methyl.

22. A compound according to claim 21, wherein R_4 is hydrogen or methyl.

23. A compound according to claim 9, wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl or substituted or unsubstituted phenyl, and Het is



24. A compound according to claim 23, wherein R_1 is substituted or unsubstituted benzyl or phenyl.

25. A compound according to claim 9 wherein X is $-S(O)_2-$.

26. A compound according to claim 25 wherein R_1 is alkyl, aryl or aralkyl.

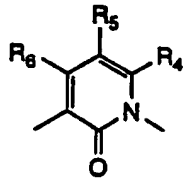
27. A compound according to claim 26 wherein R_1 is aryl or aralkyl optionally substituted with Y_1 and/or Y_2 and Y_1 and Y_2 are independently selected from $-C(O)OH$, $-C(O)OZ_1$, $-OH$, $-S(O)_mZ_1$, and $-CF_3$.

28. A compound according to claim 27 wherein R_1 is unsubstituted naphthyl, substituted naphthyl, unsubstituted phenyl, substituted phenyl, unsubstituted benzyl or substituted benzyl.

29. A compound according to claim 28 wherein R_1 is benzyl.

30. A compound according to claim 26 wherein R_1 is cyclohexyl or cyclohexylmethyl.

31. A compound according to claim 28 wherein Het is



32. A compound according to claim 9 selected from

the group consisting f:

3-[(phenylsulfonyl)amino-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(2-naphthylsulfonyl)amino]-2-oxo-1,2 dihydropyridyl-acetyl-L-argininal,

3-[(1-naphthylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-(cyclohexylaminosulfonylamino-2-oxo-1,2-dihydropyridyl)-acetyl-L-argininal,

3-(phenylaminosulfonylamino-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(phenoxycarbonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(cyclohexylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl-acetyl-L-argininal,

3-[(cyclohexylmethylsulfonyl)amino]-2-oxo-1,2 dihydro-pyridylacetyl-L-argininal,

3-[(phenethylsulfonyl)amino]-2-oxo-1,2-dihydro-pyridylacetyl-L-argininal,

3-[(2-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(3-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(4-methoxycarbonylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(2-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(3-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(4-trifluoromethylphenylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(2-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(3-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(4-methoxycarbonylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(2-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-

dihydropyridylacetyl-L-argininal,

3-[(3-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

3-[(4-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridylacetyl-L-argininal,

[3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ,

[3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1,2-dihydropyridyl]acetyl-L-argininal ,

5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinyl-acetyl-L-argininal,

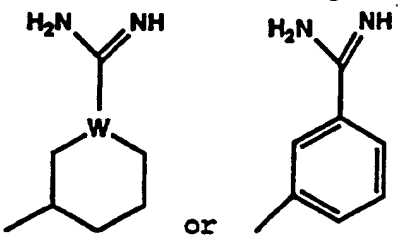
2-methyl-5-benzylsulfonylamino-6-oxo-1,6-dihydro-1-pyrimidinylacetyl-L-argininal,

5-benzylsulfonylamino-uracilylacetyl-L-argininal,

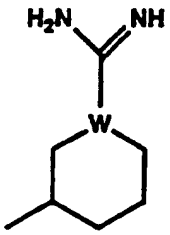
5-benzylsulfonylamino-1-methyl-uracilylacetyl-L-argininal and,

3-[(2-trifluoromethylbenzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl acetyl-L-argininal.

33. A compound according to claim 1 wherein R_3 is



34. A compound according to claim 33, wherein R_3 is



and W is nitrogren.

35. A compound according to claim 33, wherein R_4 is selected from the group consisting of:

(a) hydrogen,

(b) alkyl of 1 to 6 carbon atoms or alkyl of 1 to 6 carbon atoms substituted with Z_5, wherein Z_5 is selected from the group consisting of hydroxy, halogen, $-C(O)OH$, $-C(O)OR_8$, $-S(O)_2OH$ and $-S(O)_pR_8$,

(c) alkyl of 1 to 3 carbon atoms substituted with cyclic alkyl of 3 to 5 carbon atoms,

(d) alkenyl of about 3 to about 6 carbon atoms,

(e) cycloalkyl of about 3 to about 5 carbon atoms,

(f) heteroaryl of 5 atoms, and

(g) heteroaralkyl of 6 atoms.

35. A compound according to claim 33, wherein R_5 is selected from the group consisting of hydrogen, alkyl of 1 to about 5 carbon atoms, trifluoromethyl, and alkoxy of 1 to 4 carbon atoms.

36. A compound according to claim 35, wherein R_5 is hydrogen.

37. A compound according to claim 33, wherein R_6 is selected from the group consisting of:

(a) hydrogen,

(b) alkyl of 1 to about 12 carbon atoms or alkyl of 1 to 12 carbon atoms substituted with Z_6, wherein Z_6 is selected from the group consisting of hydroxy, halogen, $-OR_9$, $-NHR_9$, $-C(O)OH$, $-C(O)OR_9$, $-S(O)_2OH$ and $-S(O)_pR_9$.

(c) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms,

(d) alkenyl of about 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 3 to about 8 carbon atoms, or aryl of about 6 to about 10 carbon atoms,

(e) aralkyl or substituted aralkyl,

(f) heteroaralkyl of about 5 to 10 ring atoms or

substituted heteroaralkyl of about 5 to 10 ring atoms,

(g) aralkenyl of about 8 to 15 carbon atoms which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2 and/or Y_3, and

(h) heteroaralkenyl of about 5 to 10 ring atoms or substituted heteroaralkenyl of about 5 to 10 ring atoms.

38. A compound according to claim 37, wherein R_4 and R_5 are hydrogen or methyl and R_6 is selected from the group consisting of aralkyl of about 8 to about 13 carbon atoms, and -O-aralkyl, -NH-aralkyl, or $-S(O)_p$-aralkyl all of about 7 to about 12 carbon atoms.

39. A compound according to claim 38, wherein the aryl portion of the aralkyl group of R_6 is selected from unsubstituted or substituted phenyl or naphthyl.

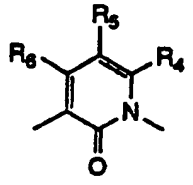
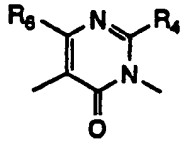
40. A compound according to claim 39, wherein said substituents of the aryl ring are selected from the group consisting of methyl, methoxy, fluoro, chloro and trifluoromethyl.

41. A compound according to claim 37, wherein R_6 is selected from the group consisting of phenylethyl, phenylpropyl, cyclohexylethyl and cyclohexylpropyl.

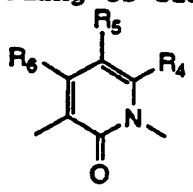
42. A compound according to claim 33, wherein R_7 is selected from the group consisting of hydrogen, methyl, difluoromethyl and trifluoromethyl.

43. A compound according to claim 42, wherein R_7 is hydrogen.

44. A compound according to claim 33, wherein Het is

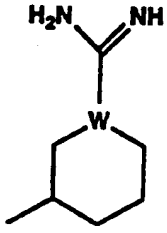
45. A compound according to claim 44, wherein Het is



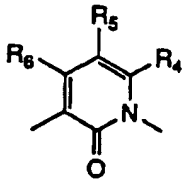
wherein R_4 is selected from the group consisting of hydrogen, methyl, ethyl, propenyl, allyl, propyl, isopropyl, butyl, R-sec-butyl, S-sec-butyl, isobutyl, 1-pentyl, R-2-pentyl, S-2-pentyl, 3-pentyl, S-1-(2-methyl)-butyl, R-2-(3-methyl)-butyl, 1-(3-methyl)-butyl, R-1-(2-methyl)-butyl, cyclopentyl, 2-pyrolyl, 3-pyrolyl, 1-hexyl, S-2-hexyl, R-2-hexyl, R-3-hexyl, and S-3-hexyl and R_5 and R_6 are independently selected from hydrogen and methyl.

46. A compound according to claim 45, wherein R_4 is hydrogen or methyl.

47. A compound according to claim 33, wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl or substituted or unsubstituted aryl, R_3 is



wherein W is nitrogen, and Het is



48. A compound according to claim 47, wherein R_1 is substituted or unsubstituted benzyl or phenyl.

49. A compound according to claim 33 wherein X is $-S(O)_2-$.

50. A compound according to claim 49 wherein R_1 is alkyl, aryl or aralkyl.

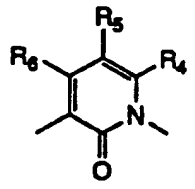
51. A compound according to claim 50 wherein R_1 is aryl or aralkyl optionally substituted with Y_1 and Y_2 and Y_1 and Y_2 are independently selected from $-C(O)OH$, $-C(O)OZ_1$, $-OH$, $-S(O)_mZ_1$, and $-CF_3$.

52. A compound according to claim 51 wherein R_1 is unsubstituted naphthyl, substituted naphthyl, unsubstituted phenyl, substituted phenyl, unsubstituted benzyl or substituted benzyl.

53. A compound according to claim 52 wherein R_1 is benzyl.

54. A compound according to claim 50 wherein R_1 is cyclohexyl or cyclohexylmethyl.

55. A compound according to claim 52 wherein Het is



56. A compound according to claim 34 wherein X is $-S(O)_2-$.

57. A compound according to claim 56 wherein R_1 is alkyl, cycloalkyl, aryl, or aralkyl.

58. A compound according to claim 57 wherein R_1 is aryl or aralkyl optionally substituted with Y_1 and Y_2 and wherein Y_1 and Y_2 are independently selected from $-C(O)OH$,

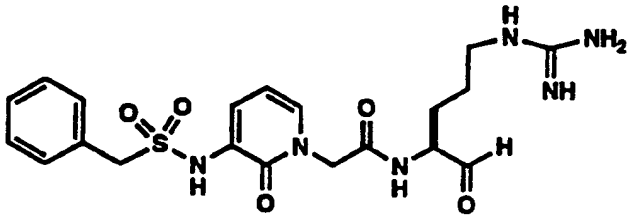
$-C(O)OZ_1$, -OH, $-S(O)_mZ_1$, and $-CF_3$.

59. A compound according to claim 58 wherein R_1 is unsubstituted naphthyl, substituted naphthyl, unsubstituted phenyl, substituted phenyl, unsubstituted benzyl, or substituted benzyl.

60. A compound according to claim 59 wherein R_1 is benzyl.

61. A compound according to claim 57 wherein R_1 is cyclohexyl or cyclohexylmethyl.

62. A compound according to claim 1 having the formula:



63. The compound [3-[(benzylsulfonyl) amino]-2-oxo-1,2-dihydropyridyl] acetyl-3-[3-piperidyl-(N-guanidino)]alaninal.

64. The compound [3-[(benzylsulfonyl)amino]-2-oxo-1,2-dihydropyridyl] acetyl-D,L-3-amidinophenyl alaninal.

65. A method of alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the compound with a solution of a zinc salt and an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with an oxidizing

agent, and

(c) recovering a 4-alkyl-3-nitro-2-oxo-1,2-dihydropyridyl acetate product.

66. The method of claim 65, wherein the zinc salt is zinc chloride or zinc bromide.

67. The method of claim 66, wherein the oxidizing agent is palladium acetate in warm THF.

68. The method of claim 65, wherein in step (a) the compound and zinc salt are first combined and then the alkyl grignard is added.

69. The method of claim 65, wherein the alkyl grignard is synthesized from a starting compound in the group defined by R_1 of formula (I).

70. The method of claim 69, wherein the alkyl grignard is 3-phenylpropyl magnesium bromide.

71. A method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the compound with zinc chloride and then adding an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with palladium acetate in warm THF, and

(c) recovering a 4-alkyl-3-nitro-2-oxo-1,2-dihydropyridyl acetate product.

72. The method of claim 71, wherein the 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound is t-butyl[3-nitro-2-oxo-1,2-dihydropyridyl]acetate and the 4-alkyl-3-nitro-2-oxo-1,2-dihydropyridyl acetate product is t-butyl [3-nitro-2-oxo-4-(3-phenylpropyl)-1,2-

dihydropyridyl]acetat .

73. A method for alkylating a 3-nitro-2-oxo-1,2-dihydropyridyl acetate compound at ring position 4 comprising

(a) combining the compound with a solution of a zinc salt and an alkyl grignard under anhydrous conditions to form a 3-nitro-2-oxo-4-alkyl-1,2,3,4-dihydropyridyl acetate intermediate,

(b) contacting the intermediate with a reducing agent, and

(c) recovering a 4-alkyl-3-amino-2-oxo-piperidyl acetate product.

74. The method of claim 73, wherein the zinc salt is zinc chloride or zinc bromide.

75. Te method of claim 73, wherein the reducing agent is hydrogen.

76. The method of claim 73, wherein in step (a) the compound and zinc salt are first combined and then th ealkyl grignard is added.

77. The method of claim 73, wherein the alkyl grignard is synthesized from a starting compound in the group defined by R_1 in formula (I).

Figure 1

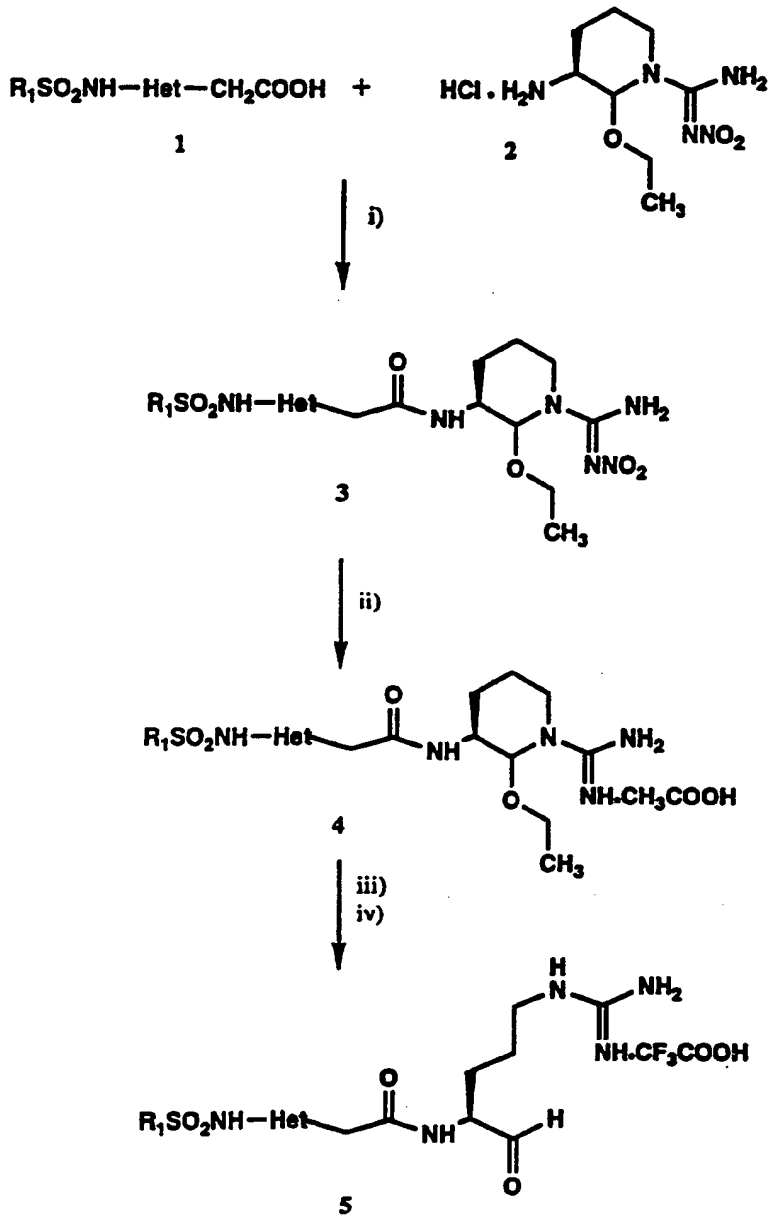

R1SO2NH—Het—CH2COOH + HCl . H2N
1
2
NH2
NNO2
O
CH3
i)
R1SO2NH—Het
NH
3
ii)
NH-CH3COOH
4
iii)
iv)
NH-CF3COOH
H
5

Figure 2

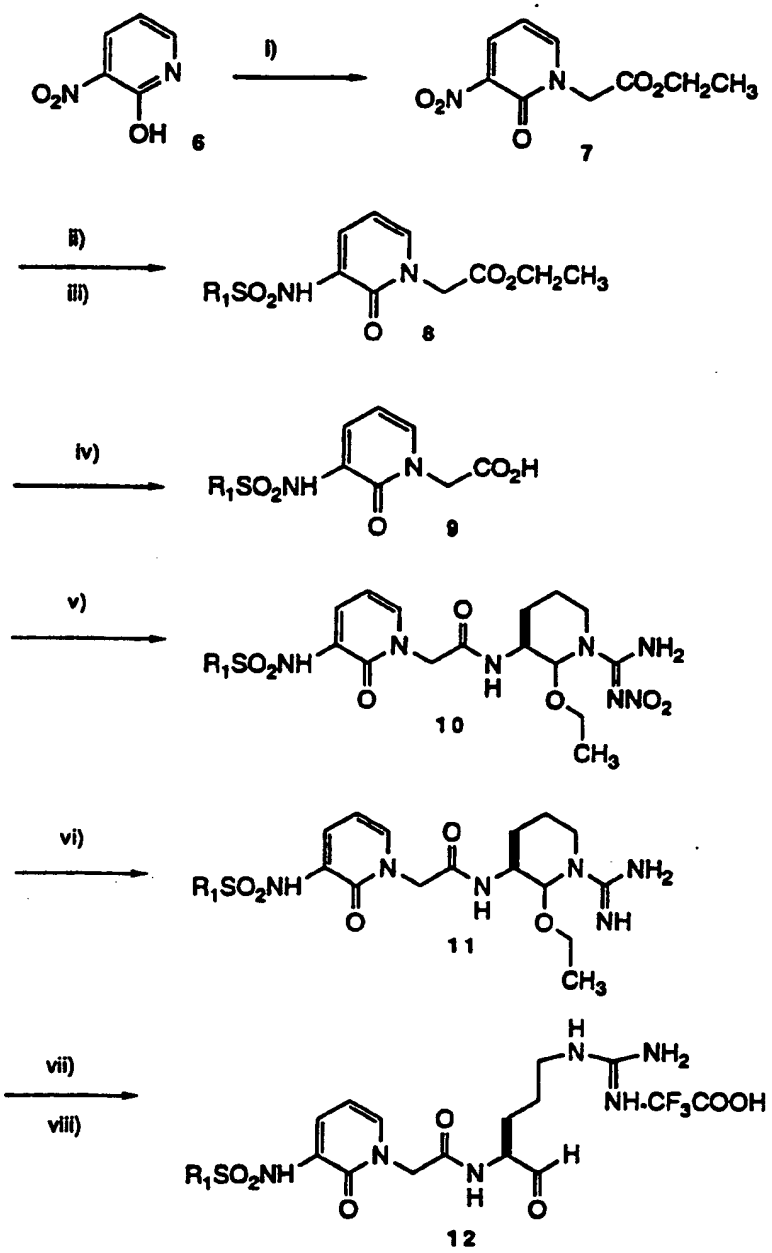


Figure 3

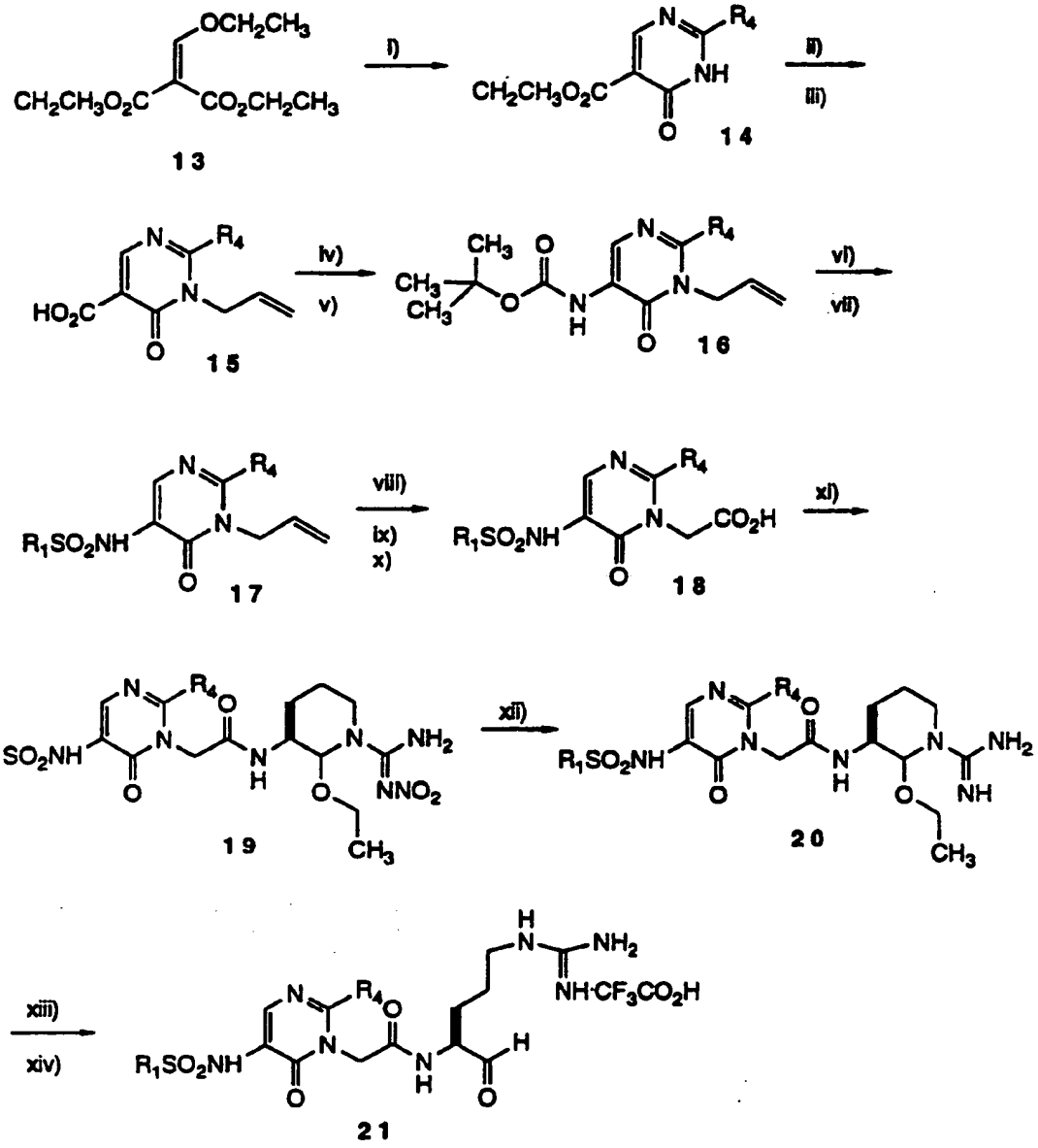

i)
ii)
iii)
iv)
v)
vi)
vii)
viii)
ix)
x)
xi)
xii)
xiii)
xiv)
13
14
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Figure 4

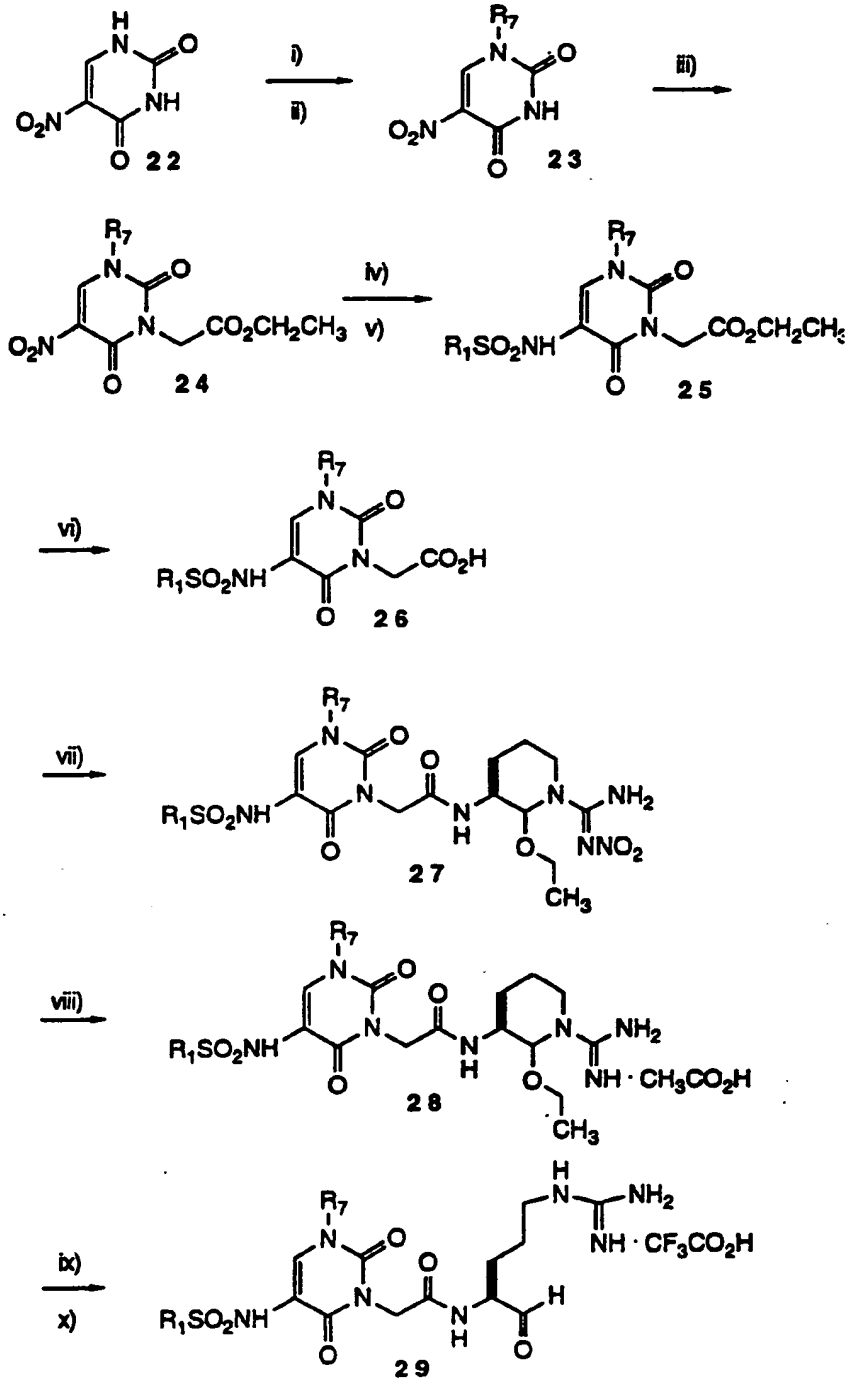


Figure 5

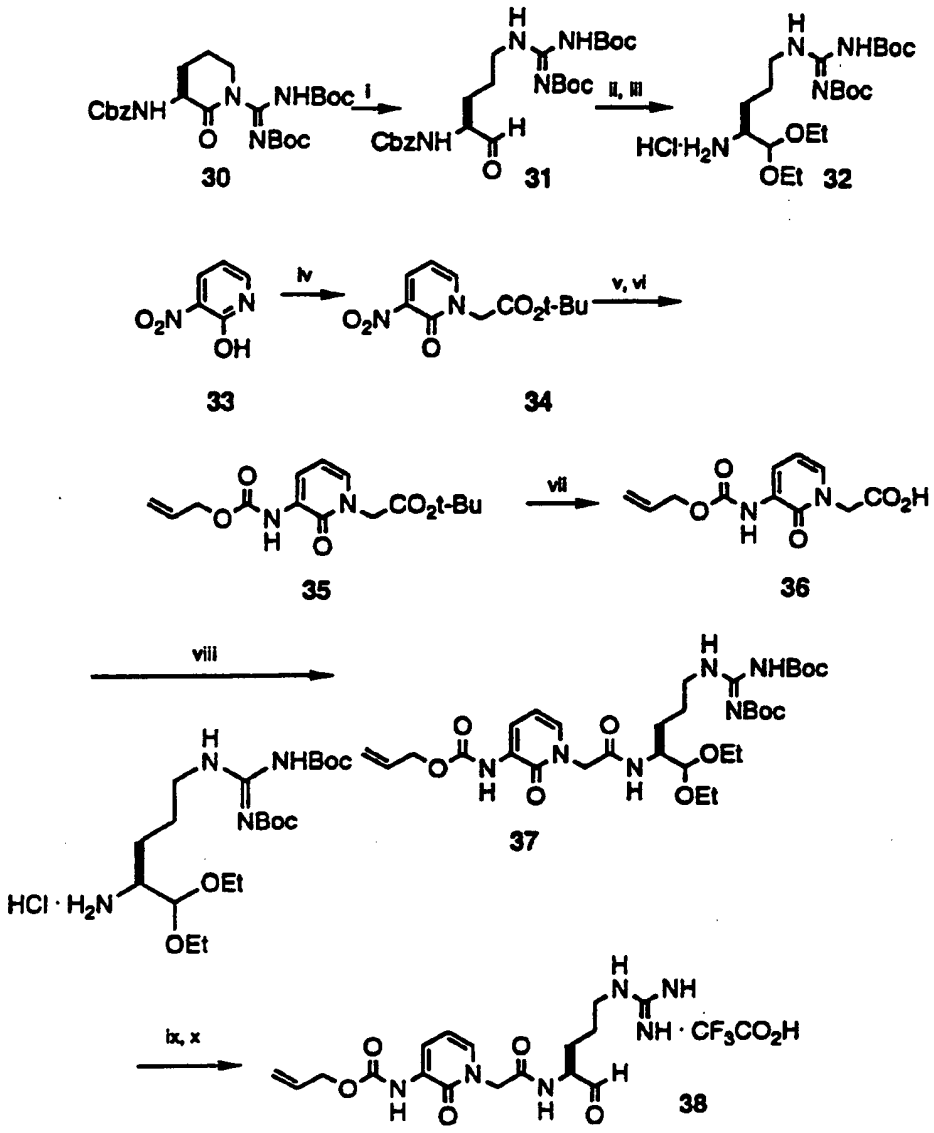

CbzNH
NHBoc
NBoc
i
30
31
ii, iii
HCl·H2N
OEt
OEt
32
iv
O2N
OH
33
CO2t-Bu
v, vi
34
vii
CO2H
35
36
viii
37
ix, x
NH · CF3CO2H
38

Figure 6

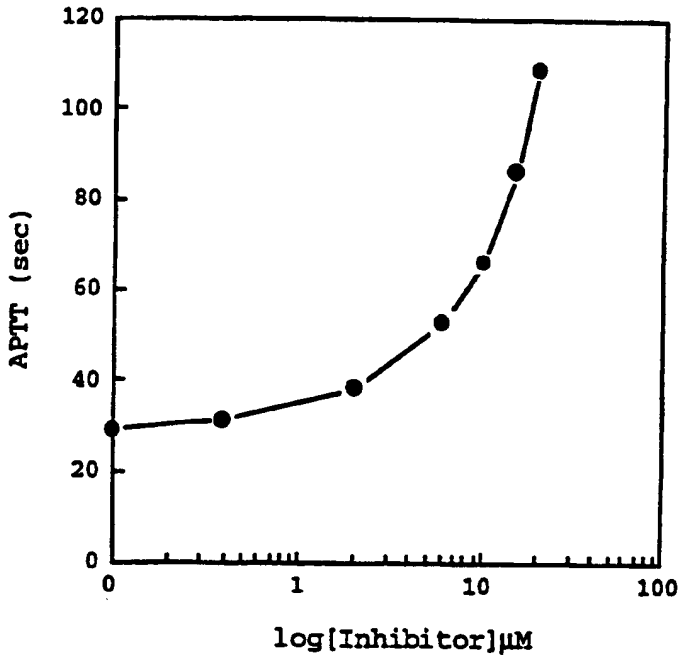


Figure 7

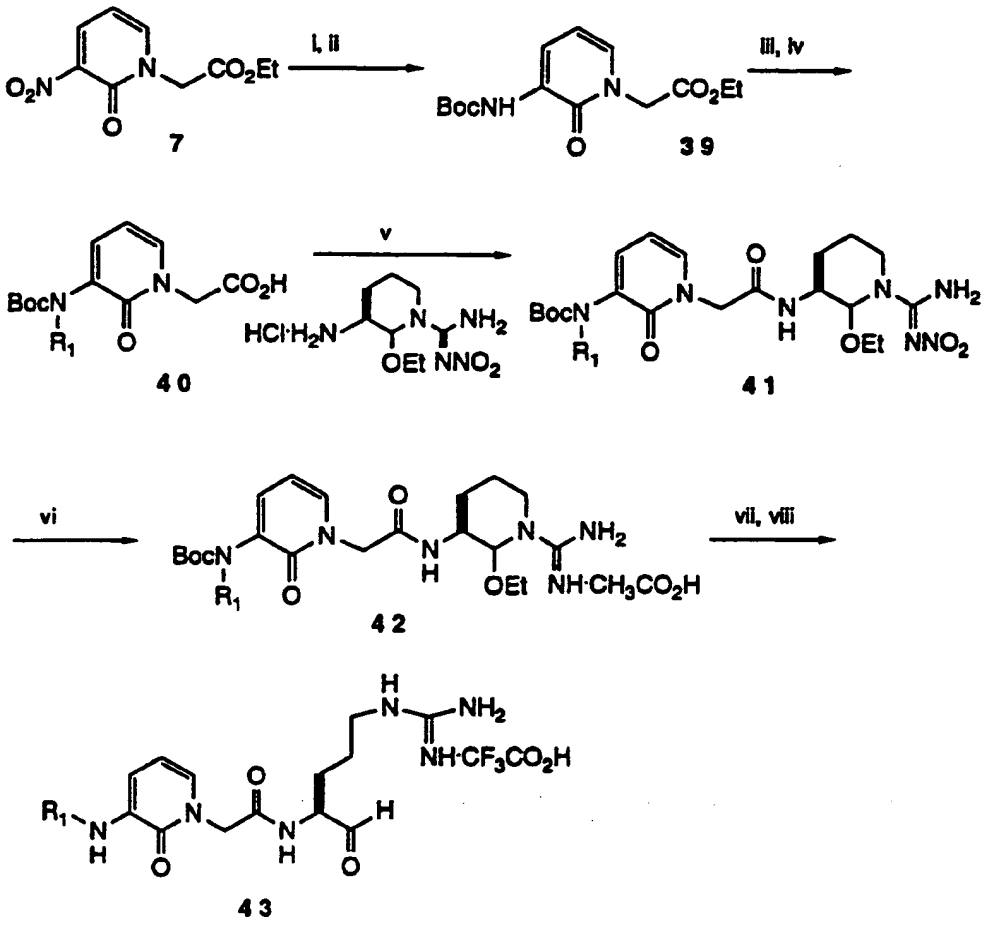
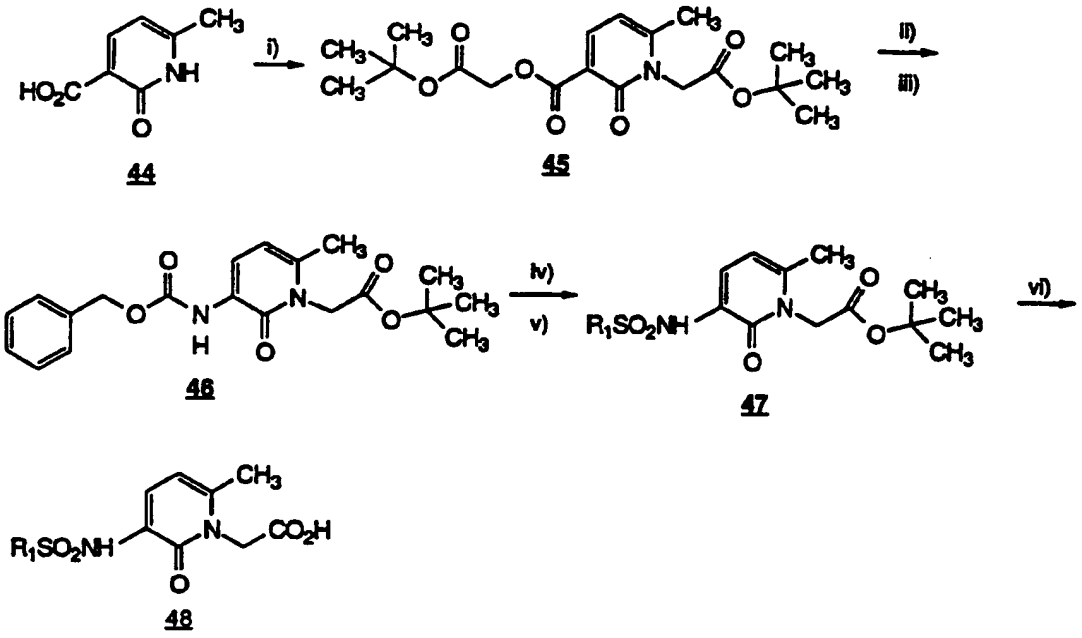
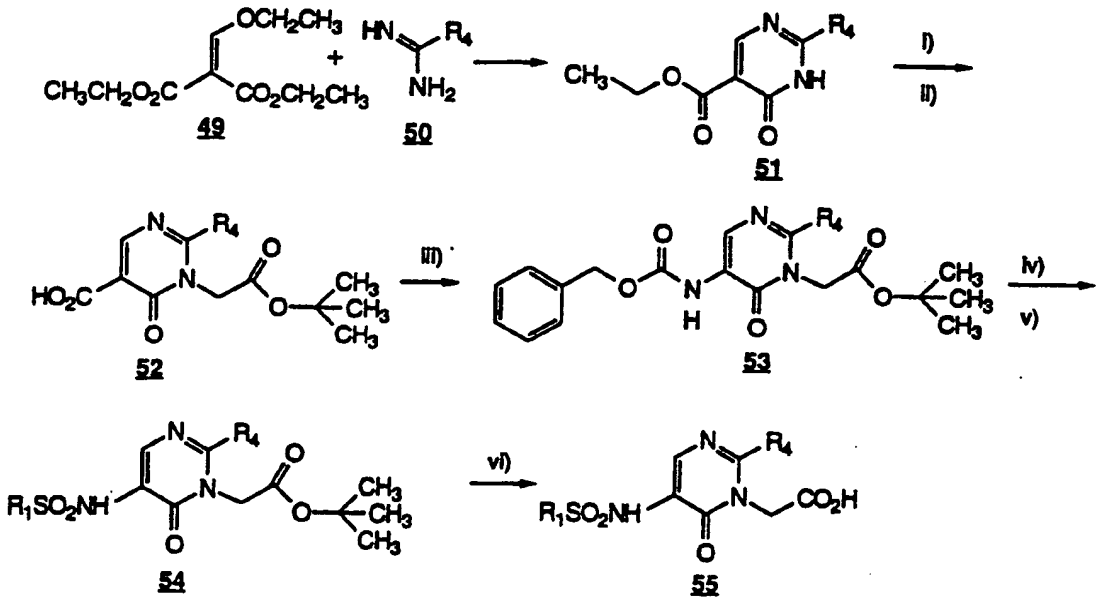


Figure 8



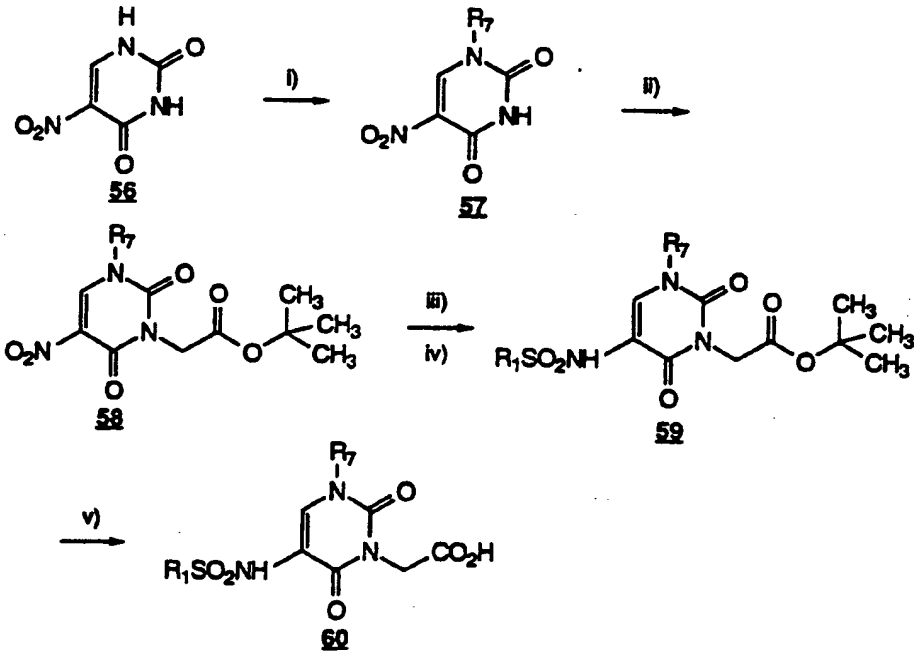
i) K_2CO_3, DMF, $BrCH_2CO_2t$-Bu; ii) LiOH, THF; iii) Et_3N, DPPA, dioxane, Δ; BnOH, Δ; iv) H_2, Pd/C; v) R_1SO_2Cl, collidine; vi) TFA

Figure 9



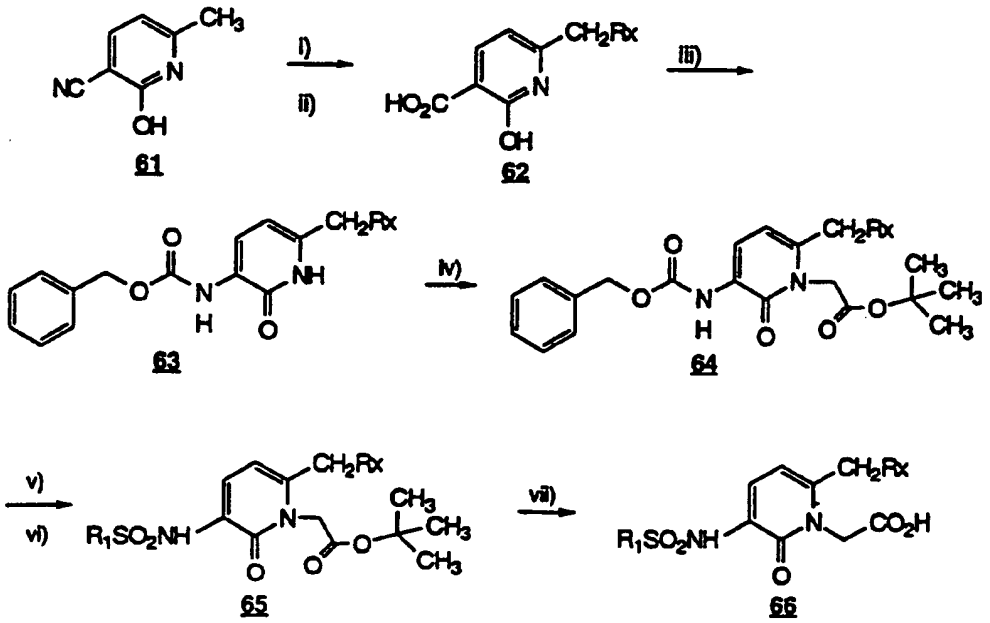
i) NaH, DMF, $BrCH_2CO_2t$-Bu; ii) LiOH, THF; iii) Et_3N, DPPA, dioxane, Δ; BnOH, Δ; iv) H_2, Pd/C; v) R_1SO_2Cl, NMM; vi) TFA

Figure 10



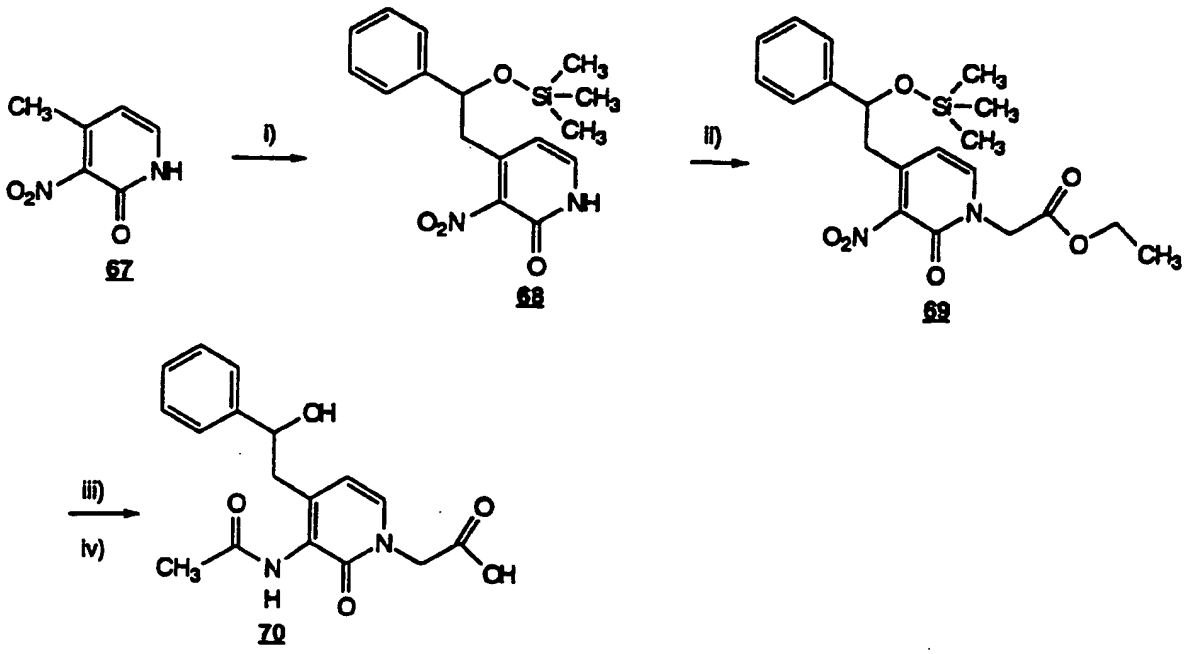
i) K_2CO_3, R_7X, DMSO; ii) NaH, $BrCH_2CO_2t$-Bu: iii) H_2, 10% Pd/C; iv) R_1SO_2Cl, NMM; v) trifluoroacetic acid

Figure 11



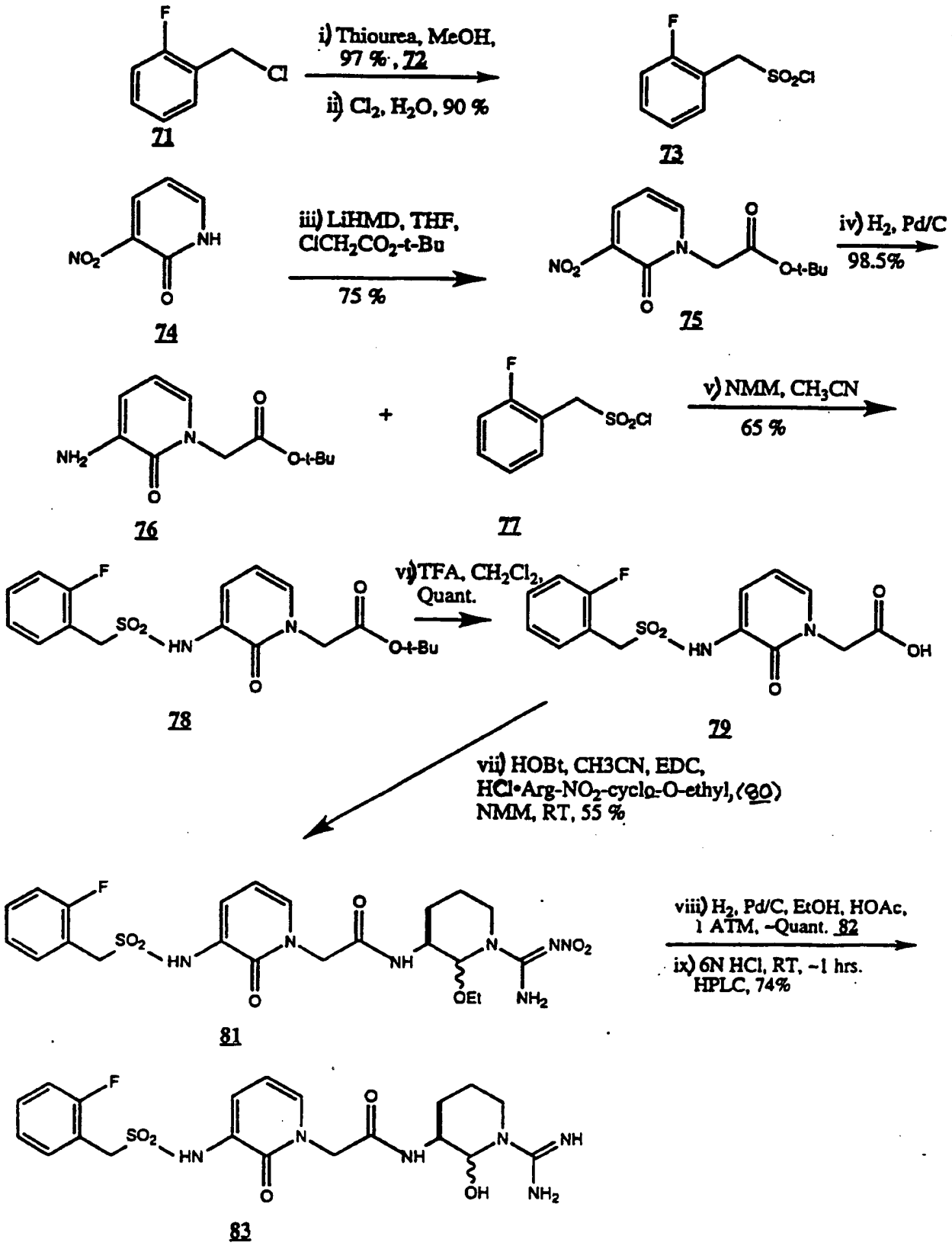
i) 2 equiv. LDA; R_xX; 50% H_2SO_4; iii) Et_3N, DPPA, dioxane, Δ; BnOH, Δ; iv) NaH, DMF, $BrCH_2CO_2t$-Bu
v) H_2, Pd/C; vi) R_1SO_2Cl, collidine; vii) TFA

Figure 12



i) $LiN(TMS)_2$; TMSCl; $LiN(TMS)_2$; benzaldehyde; ii) $LiN(TMS)_2$, ethyl bromoacetate; iii) Ac_2O, 10% Pd/C, H_2, iv) LiOH

Figure 13


i) Thiourea, MeOH, 97 %, 72
ii) Cl2, H2O, 90 %
iii) LiHMD, THF, ClCH2CO2-t-Bu
75 %
iv) H2, Pd/C
98.5%
v) NMM, CH3CN
65 %
vi) TFA, CH2Cl2, Quant.
vii) HOBt, CH3CN, EDC, HCl•Arg-NO2-cyclo-O-ethyl, (80) NMM, RT, 55 %
viii) H2, Pd/C, EtOH, HOAc, 1 ATM, ~Quant. 82
ix) 6N HCl, RT, ~1 hrs. HPLC, 74%
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US 95/16410

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 A61K31/44 A61K38/05 C07D213/76 C07D213/75
C07D239/54 C07D401/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 17817 (CORVAS INT INC) 18 August 1994 see the whole document -----	1-64

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 1996	15 -05- 1996

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	De Jong, B

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US 95/16410

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9417817	18-08-94	CA-A-	2155931	18-08-94
		EP-A-	0684830	06-12-95
		US-A-	5492895	20-02-96

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International Bureau

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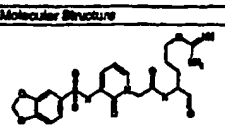
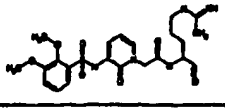
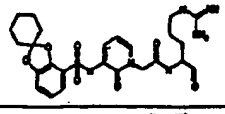
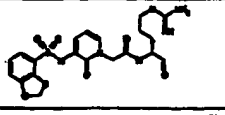
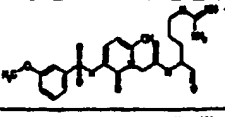
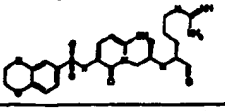
(54) Title: AROMATIC HETEROCYCLIC DERIVATIVES AS ENZYME INHIBITORS

(57) Abstract

The present invention discloses peptide aldehydes which are potent and specific inhibitors or thrombin, their pharmaceutically acceptable salts, pharmaceutically acceptable compositions thereof, and methods of using them as therapeutic agents for disease states in mammals characterized by abnormal thrombosis.

Example number	Molecular name (abbreviated)	Molecular Structure
1528A		
1528B		
1528C		
1528D		
1528A		
1528B		
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